Herpetological Conservation and Biology 6(3):432–442.
Submitted: 12 July 2010; Accepted: 30 August 2011; Published: 31 December 2011.

MORPHOLOGICAL ABNORMALITIES IN AMPHIBIAN POPULATIONS FROM THE MID-EASTERN REGION OF ARGENTINA

PAOLA M. PELTZER[1], RAFAEL C. LAJMANOVICH[1], LAURA C. SANCHEZ[1], ANDRÉS M. ATTADEMO[1], CELINA M. JUNGES[1], CLARISA L. BIONDA[2], ADOLFO L. MARTINO[2], AND AGUSTÍN BASSÓ[1]

[1] National Council for Scientific and Technical Research (CONICET) - Faculty of Biochemistry and Biological Sciences, National University of Litoral, Paraje el Pozo s/n (3000), Santa Fe, Argentina,
e-mail: paolapeltzer@hotmail.com; lajmanovich@hotmail.com
[2] Department of Natural Sciences, National University of Río Cuarto, Roads 8, Km 601, (5800) Río Cuarto, Cordoba, Argentina. e-mail: amartino@exa.unrc.edu.ar

***Abstract.*—We present the first compilation and analysis of cases of morphologically abnormal anurans from the mid-eastern region of Argentina (Córdoba, Santa Fe, and Entre Ríos Provinces). We sampled for abnormal individuals at 51 sites in agricultural, suburban, and forest settings between January 2000 and December 2009. We recorded 71 abnormal individuals, including 16 types of abnormalities in 15 anuran species. In agricultural sites, we found 12 types of abnormalities, with ectromelia being the most abundant. In suburban sites, we recorded seven types of abnormalities, with brachygnathia and ectromelia being the most common. In forest sites, we found three types of abnormalities, with ectromelia again being the most abundant. This study is the first catalog of anurans with morphological abnormalities in Argentina, thus expanding the geographic range of observed abnormalities in amphibians and illustrating the ubiquity of this phenomenon.**

Key Words.—abnormal anurans; agroecosystems; ectromelia; native forests; suburban areas

ANORMALIDADES MORFOLÓGICAS EN POBLACIONES DE ANFIBIOS DE LA REGIÓN CENTRO-ESTE DE ARGENTINA

***Resumen.*—Se presenta la primer compilación y análisis de casos de anuros morfológicamente anormales de la región centro-este de Argentina (Provincias Córdoba, Santa Fe, y Entre Ríos). Los anuros anormales fueron recolectados en agroecosistemas, suburbios, y sitios de referencia, desde enero de 2000 hasta diciembre de 2009. Se registraron 71 individuos anormales, comprendiendo 16 tipos de anormalidades en 15 especies de anuros. En los sitios agrícolas se encontraron 12 tipos de anormalidades, siendo ectromelia la más abundante. En los sitios suburbanos se registraron siete tipos de anormalidades, siendo braquignatia y ectromelia las más comunes. En los sitios de referencia también se encontraron tres tipos de anormalidades, y ectromelia resultó la más abundante. Aunque el diseño de nuestro limita el número de conclusiones que pueden obtenerse, este es el primer catálogo de anuros con anormalidades morfológicas en Argentina, proporcionando datos de referencia y estableciendo criterios para monitorear las anormalidades de anuros en diferentes áreas. Además, este estudio amplía el ámbito geográfico de las anormalidades observadas en los anfibios e ilustra la ubicuidad de este fenómeno.**

Palabras Claves.—agroecosistemas; anuros anormales; áreas suburbanas; bosques nativos; ectromelia

INTRODUCTION

Reports of amphibians exhibiting morphological abnormalities come from diverse biomes around the world (Ouellet 2000). Surprising numbers of malformed anurans have been found in North America, as well as several countries in Asia, Europe, and Australia (e.g., Voitkevich 1961; Read and Tyler 1990; Takeishi 1996; Vandenlangenberg et al. 2003; Piha et al. 2006). However, studies are lacking from South America, where the literature contains descriptions of only a few malformed species (e.g., Prigione and Langone 1985; Peri and Williams 1988; Massemin and Marty 2000). In Argentina, observations of abnormalities have been episodic and were recorded affecting a single species in restricted locations (e.g., Peri and Williams 1988; Fabrezi 1999). Although reports of abnormal anurans exist from several native forests, wetlands, and agroecosystems (Peltzer et al. 2001; Attademo et al. 2004), no recent inventories or analyses of abnormal amphibians are available. In this sense, limited historical data on morphological abnormalities combined with the absence of baseline abnormalities data could complicate attempts to assess whether malformations are emerging.

The purpose of this study was to analyze and describe cases of morphological abnormalities of anurans from three Provinces (Córdoba, Santa Fe, and Entre Ríos) located in the mid-eastern region of Argentina, an area dominated by agricultural landscapes. This study also provides baseline data to monitor anuran abnormalities in different areas of Argentina and expands the number of known regions of the world from which amphibian malformations have been reported (e.g., Gurushankara et al. 2007).

MATERIALS AND METHODS

***Study area.*—**We sampled abnormal anurans from three types of sites (agricultural sites [N = 22], suburban sites [N = 16], and forest sites [N = 13]) distributed in three Provinces (Córdoba, Santa Fe, and Entre Ríos) from the mid-eastern region of Argentina (Fig. 1). Fluvial forest, wetlands, and temperate steppe vegetation historically dominated this region (Burkart et al. 1999). The traditional dairy-oriented agriculture that was characterized by a mosaic of pastures and woodlots has been replaced by the large-scale production of genetically modified (GM) Roundup Ready (RR) soybeans, and to a lesser extent, rice, corn, and sorghum (Aizen et al. 2009). For example, the area planted with soybean has increased rapidly since the 1970s, from < 1 million ha in 1970/71, to 6.3 million ha in 1996/97 (the year when GM soybean was introduced) and 16.6 million ha in 2007/08 (Food and Agriculture Organization. 2011. FAOSTAT. Food and Agriculture Organization, Rome. Available from http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567 [Accessed August 2009]). This area has continued growing, reaching 18.1 million ha in 2009/10, accounting for about 60% of the surface cultivated in Argentina (Secretaria de Medios de Comunicación. 2010. Domínguez: somos el primer exportador mundial de aceite de soja. Sala de prensa. Agricultura. Available from http://www.prensa.argentina.ar/2010/10/09/12736.php [Accessed December 2010]).

In our study, agricultural sites were sites in which soybean or rice had been cultivated for at least two years prior to the start of the amphibian surveys. In these sites, agricultural runoff is a significant source of pollution of aquatic environments (Peltzer et al. 2008). Suburban sites were ephemeral and semipermanent ponds that were located within 15 km of urban areas. Human activities affect the quality of suburban bodies of water because the water may be used for waste disposal and because there may be insufficient drainage of sewage (Massone et al. 1998). The forest sites we surveyed were fragments of native vegetation with a flora and fauna similar to those described by Burkart et al. (1999) from the Deltas and Islands of Paraná River and Espinal ecoregions. They were natural reserves or private areas with water bodies that differed in hydroperiod (ephemeral, semipermanent, and permanent), and we considered them references for comparisons to sites more disturbed by human activities. These sites were located at distances > 10 km from an urban metropolis and from any agroecosystems. It is important to note that these agricultural, suburban, and forest sites have different compositions of anuran species (Lajmanovich and Peltzer 2001; Peltzer et al. 2003, 2006; Peltzer and Lajmanovich 2007).

***Anuran collection and abnormality analysis.*—**We collected animals between January 2000 and December 2009 during continuous research efforts to study the biology and ecotoxicology of anurans in different habitats (agricultural, forest, and suburban sites) in the mid-eastern region of Argentina (e.g., Lajmanovich 2000; Peltzer et al. 2003, 2006; Attademo et al. 2005; Lajmanovich et al. 2008). We captured live anurans using pitfall trap lines (Greenberg et al. 1994) or by hand using headlamps in nocturnal searches (a combination of the visual encounter surveys and audio strip transects as described in Crump and Scott [1994] and Zimmerman [1994]). We used these techniques primarily for the purposes of the studies cited above, but also to record cases of abnormal anurans (this study). We conducted standardized field surveys at least three times in each site in the springs and summers from September through April, synchronous with the major anuran feeding and breeding activities. We used a minimum effort of approximately six person-hours per sampling in each site. We geo-referenced the location of each abnormal amphibian using a global positioning system (GPS; Garmin eTrex® Navigator; Garmin Corporation, Olathe, Kansas, USA) and then transported the specimen to the lab for analysis. We took digital photos, and then we released the animals at the site of collection, except those animals that presented serious morphological abnormalities (e.g., brachygnathia, or abnormal shortness of lower jaw with tongue absence). We euthanized and fixed in 10% formalin the latter individuals (ASIH, HL, and SSAR. 2001. Guidelines for use of live amphibians and reptiles in field research. Available from http://www.research.ucf.edu/Compliance/pdf/HERPC~21.HTM [Accessed 13 January 2010]), and deposited them into amphibian collections of a museum or laboratory. We only recorded the number of morphologically abnormal amphibians in each type of site observed (agricultural, suburban, and forest sites).

We considered only abnormalities visible by eye, reporting substantial deviations from the general body plan (following Johnson et al. 2001 and Gurushankara et al. 2007). We followed Meteyer (2000) and Lannoo (2008) criteria for abnormality classifications and their respective definitions (Table 1). In addition, we described new types of abnormalities. We did not include morphological abnormalities that require detection via radiography or clearing and staining. As a result, our study may underestimate the true number of abnormalities in these populations. Moreover, we acknowledge that the difference between traumatic and developmental abnormalities is not always immediately evident (Johnson et al. 2001). In this sense, we adopted a conservative

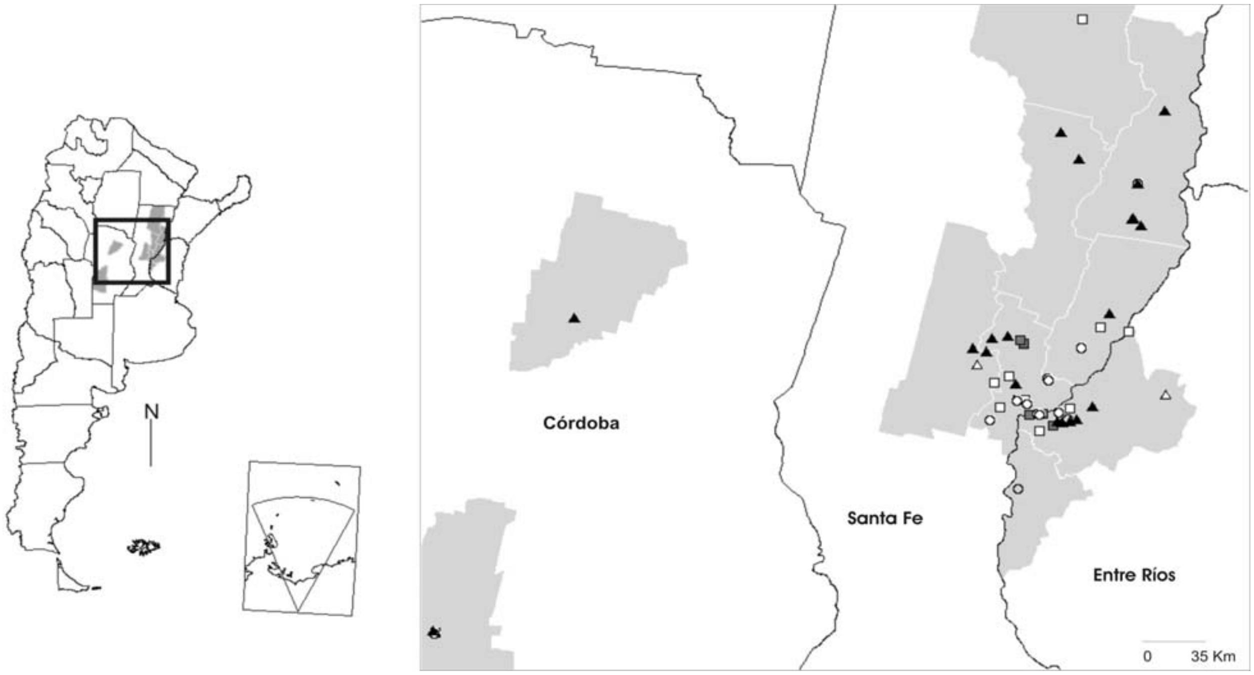

FIGURE 1. Location of agricultural (△; ▲), suburban (□; ■), and forest sites (○; ●) studied in Córdoba, Santa Fe, and Entre Ríos Provinces (Argentina). Filled symbols indicate the presence of individuals with morphological abnormalities, solid lines show province boundaries, and divisions in shaded areas represent departmental boundaries.

position and did not use the term "malformation," which is more specific and includes only those abnormalities that arise from abnormal development.

Data analysis.—We calculated the relative percentage of individuals with abnormalities by total area analyzed, types of sites, and species. Similarly, for each type of site, we calculated the frequency of each type of abnormality as the percentage of individuals with each type of abnormality relative to the total number of abnormal individuals examined (Johnson et al. 2002). We did not present data on the prevalence of abnormalities within a population because our field surveys were not designed for that type of quantitative comparison (i.e., we did not have data on all normal and abnormal individuals to calculate frequencies, but only had data on numbers of abnormal individuals). Using G tests ($\alpha = 0.05$) of independence (Sokal and Rohlf 1995), we tested for associations between the presence of abnormalities (sum of all types) and types of sites (agricultural, suburban, and forest sites). Similarly, we performed another G test to examine associations between the presence of the most common abnormality (ectromelia) and types of sites.

RESULTS

We identified 71 abnormal individuals from 51 sites, including 16 types of abnormalities in 15 anuran species (Table 1). Some of the most commonly observed abnormalities are illustrated in Fig. 2. Abnormalities were generally distributed throughout the study area (Fig. 1). Most morphological abnormalities involved ectromelia (49.29%, missing limb segments), amelia (8.45% missing limb), brachygnathia (7.04%, abnormal shortness of lower jaw), and forked tail (7.04%; Table 1, Fig. 3). Moreover, phocomelia (absence of the proximal portion of a limb with the foot attached very close to the body), absence of horny covering in eyes, missing tympanum, hemimelia (shortened bone), ectrodactyly (missing toe), brachydactyly (short toe), forelimb remaining under skin, polydactyly (extra digit), polymelia (complete extra limb), syndactyly (fusion of digits), alterations in the back skin, and ulcerated skin combined represented fewer than 28.2% of all abnormal anurans.

Morphological abnormalities and type of sites.—There was a significant difference (G test = 22.58, df = 2, $P < 0.005$) in the presence of abnormalities among the different types of sites (90.90% in agricultural sites; 43.75% in suburban sites, and 15.38% in forest sites). We found 12 types of abnormalities in the different agricultural sites sampled (Table 1). Ectromelia was the most common (54.76%), followed by amelia (14.28%), and then by lower relative percentages (<3%) for absence of a horny covering, missing tympanum, brachydactyly, phocomelia, polydactyly, syndactyly, and alterations in the back skin. Seven type of abnormalities were distributed among the suburban sites (Table 1), with brachygnathia being the most common (26.31%), followed by ectromelia (21.05%), and then forked tail

Table 1. Anuran morphological abnormalities from agricultural, suburban, and forest sites from the mid-eastern region of Argentina. Values indicate the relative percentage of each type of abnormality with respect to the total number of abnormalities in category, with the number for each abnormality listed in parentheses.

		Relative percentage of all morphological abnormalities (n)			
Type of Abnormality	Description	Agricultural sites	Suburban sites	Forest sites	All sites
Craniofacial					
Absence of horny covering in eyes		2.38 (1)	–	–	1.40 (1)
Brachygnathia**	abnormal shortness of lower jaw		26.32 (5)	–	7.04 (5)
Missing tympanum	Lack of tympanic membrane	2.38 (1)	–	10.00 (1)	2.81 (2)
Limbs					
Amelia*	completely missing limb	14.28 (6)	–	–	8.45 (6)
Brachydactyly	short toe: normal number of metatarsals but abnormal number of phalanges	2.38 (1)	–	–	1.40 (1)
Ectromelia	Complete missing limb segment	54.76 (23)	21.05 (4)	80.00 (8)	49.29 (35)
Forelimb remaining under skin		4.76 (2)	5.26 (1)	–	4.22 (3)
Hemimelia	shortened bone	4.76 (2)	–	–	2.81 (2)
Phocomelia	absence of the proximal portion of a limb, with the foot attached very close to the body	2.38 (1)	10.52 (2)	–	4.22 (3)
Polydactyly	extra digit, including metatarsal bone	2.38 (1)	–	–	1.40 (1)
Polymelia	complete extra limb	–	10.52 (2)	–	2.81 (2)
Syndactyly	fused digits	2.38 (1)	–	–	1.40 (1)
Mixed abnormalities: Hemimelia + ectrodactyly + brachydactyly	missing toe: completely missing digit including the metatarsal bone and phalanges	–	–	10.00 (1)	1.40 (1)
Others					
Alterations in the dorsal skin		2.38 (1)	–	–	1.40 (1)
Forked tail *	bifurcated tail	4.76 (2)	15.78 (3)	–	7.04 (5)
Ulcerated skin		–	10.52 (2)	–	2.81 (2)
Number of individuals with abnormalities		42	19	10	71
Number of sites with abnormal individuals		20	7	2	29
Number of inspected sites		22	16	13	51
Number of species with abnormalities		12	5	4	15
Number of type of abnormalities		12	7	3	16

* indicates tadpole stage
** indicates juvenile stage

(15.78%). We found three types of abnormalities among the forest sites, with ectromelia (80%) being the most common abnormality (Table 1). Overall, ectromelia was observed in the 29.40% (N = 15) of all studied sites. The presence of ectromelia was associated with type of site (G test = 8.79, df = 2, $P < 0.05$), being most frequently observed in agricultural sites (50%, N = 11 sites), compared with 18.75% (N = 3) of suburban sites and 7.69% (N = 1) of forest sites.

Morphological abnormalities and anuran species.— There were 12 species with abnormalities in agricultural sites, compared with five in suburban and four in forest sites. We observed abnormal *Rhinella fernandezae* and *Leptodactylus latrans* in all types of sites, while abnormal *R. arenarum*, *L. chaquensis*, and *Hypsiboas pulchellus* occurred in both suburban and agricultural sites.

In agricultural sites, *R. arenarum* and *L. latinasus* were the species with the greatest percentages of abnormalities (16.6%, respectively), followed by *R. fernandezae* (11.9%), *R. schneideri* (9.5%), and *L. chaquensis* (9.5%). In suburban sites, *H. pulchellus* and *L. latrans* (26.3% each) presented the highest abnormality percentages. Finally, in forest sites, *R. fernandezae* accounted for 50% of the abnormalities,

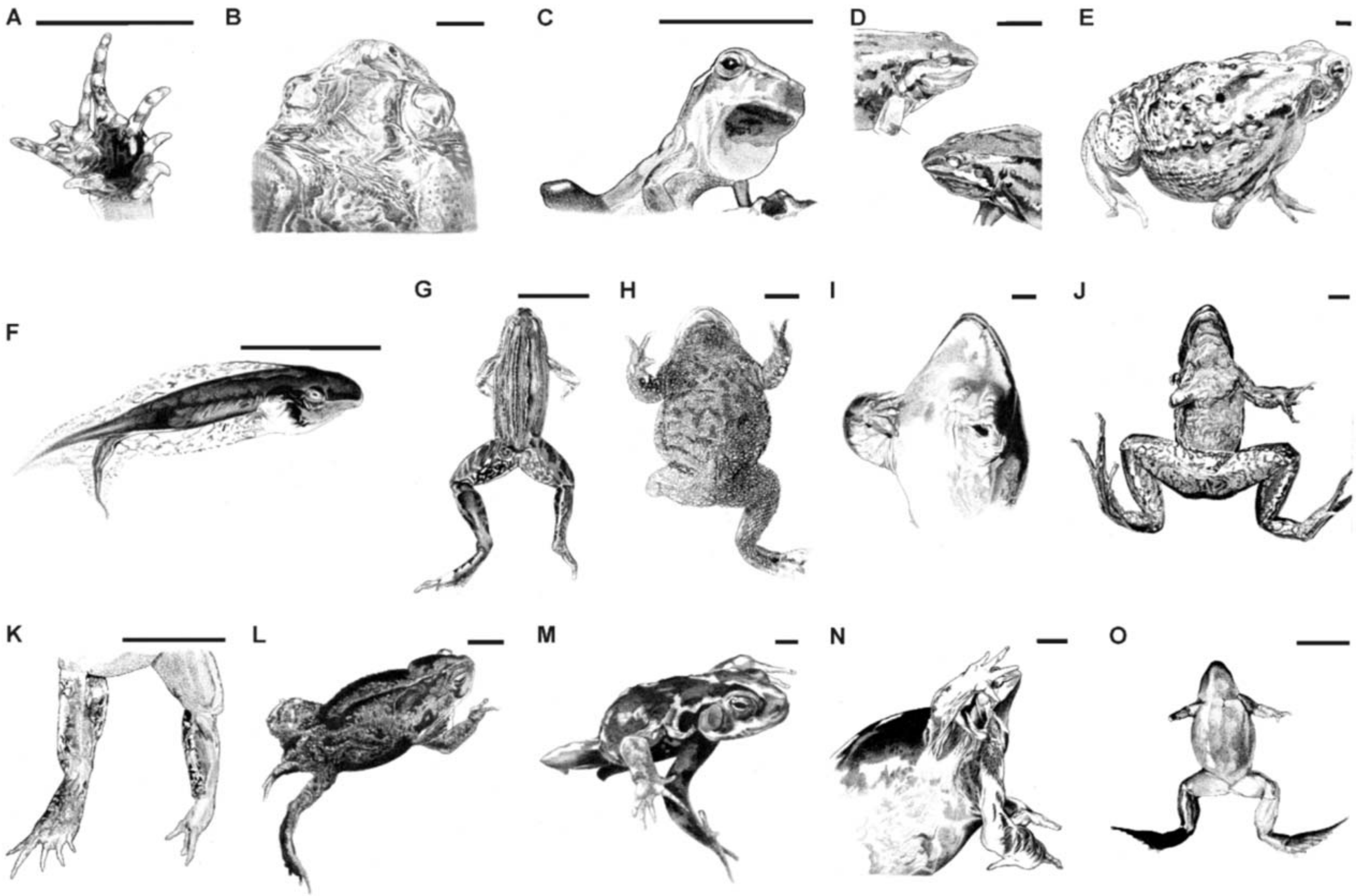

FIGURE 2. Types of abnormalities recorded in the mid-eastern region of Argentina. A) Polydactyly of forelimb in *P. biligonigerus*. B) Absence of horny covering in *R. schneideri*. C) Brachygnathia in *H. pulchellus* juvenile. D) Missing tympanum in *L. mystacinus*. E) Ectromelia of the humerus in *R. schneideri*. F) Forked tail in *S. nasicus* tadpole. G) Ectromelia of the tibiale and fibulare in *L. latrans* juvenile. H) Ectromelia of the femur in *R. fernandezae*. I), J) Forelimb remains under skin in *L. latrans*. K) Hemimelia + Ectrodactyly + Brachydactyly in *L. mystacinus*. L) Polymelia in *R. fernandezae.* M), N) Polymelia in *R. arenarum*. O). Ectromelia of the radio-ulna in *E. bicolor*. Bar = 10 mm.

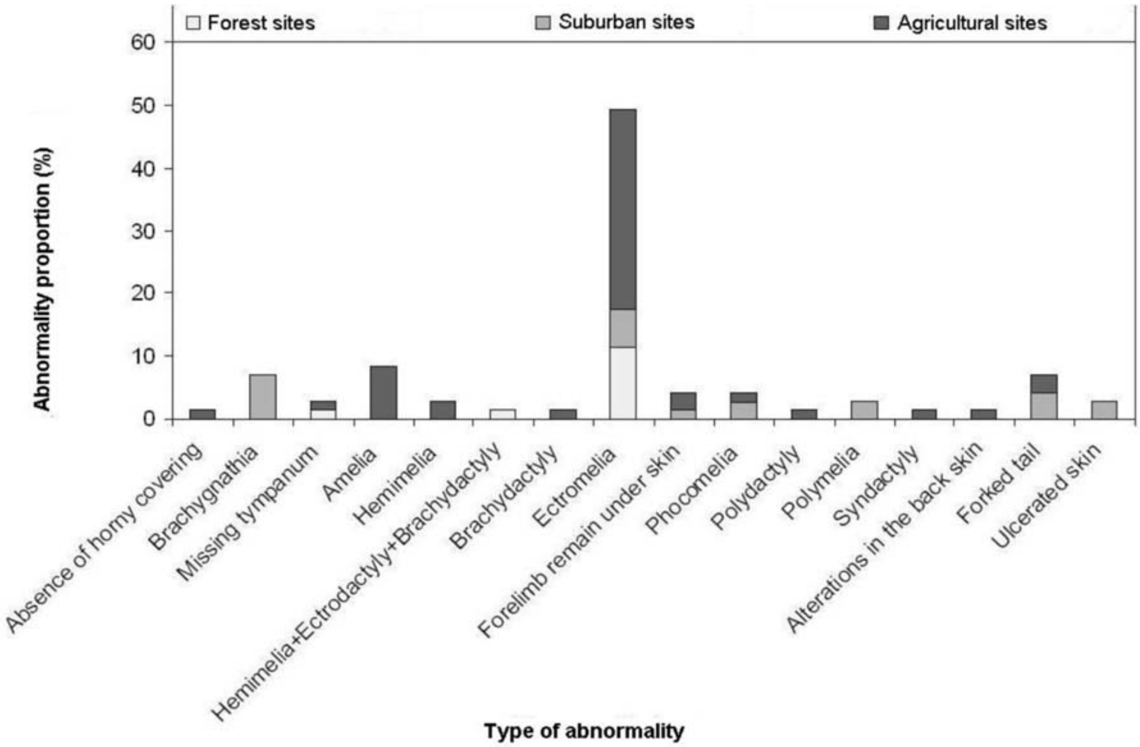

FIGURE 3. Morphological abnormality percentages based on the total number of abnormal individuals (N = 71) recorded within agricultural, suburban, and forest sites from the mid-eastern region of Argentina.

followed by *L. latrans* and *L. mystacinus* (each accounting for 20% of the abnormalities).

DISCUSSION

Although our study has limitations in interpretation because we only recorded abnormal individuals and did not sample the same number of sites among the three types of sites, this is the first catalog of anurans with morphological abnormalities for Argentina. In addition, this study expands the geographic range of observed abnormalities in amphibians and illustrates the ubiquity of this phenomenon. While our comparisons between sites are preliminary for the reasons discussed above, we noted that the number of abnormal individuals in agricultural sites was four times higher than in forest sites.

Although we did not investigate the causes of the observed abnormalities, reported causes of abnormalities among amphibians include trematode parasites (Sessions and Ruth 1990; Johnson et al. 1999, 2004), predation (Eaton et al. 2004; Ballengée and Sessions 2009), ultraviolet (UV-B) radiation (Ankley et al. 2000, 2002), and chemical pollutants (Gardiner and Hoppe 1999; Taylor et al. 2005; Skelly et al. 2007; Lannoo 2008). The ability to recognize the proximate cause(s) of abnormalities is essential because of the light it may cast on environmental degradation as well as amphibian disease and decline (Ballengée and Sessions 2009). Researchers should consider several points in future studies that are relevant to the situation in Argentina. First, a recent report (Hamann and Gonzalez 2009) about digenetic trematodes in anuran tadpoles in Argentina suggests an insufficient connection between these and amphibian limb abnormalities. However, this trematode was reported by Ostrowski de Nuñez (1968), Labriola and Suriano (1998), and Lunaschi et al. (2007) in lakes from Buenos Aires Province (Argentina) in definitive hosts such as *Phalacrocorax olivaceous* and *Ardea alba*, two common aquatic birds of which the latter is an important consumer of amphibians (Beltzer et al. 2010). We are aware that a systematic study of the trematode-limb abnormality relationship is necessary in Argentina. Such a study would need to include examination of the recently published hypotheses regarding differences in amphibian species susceptibility (Johnson et al. 2008; Johnson and Hartson 2009) and the interactions of pesticides with parasite infections (Kiesecker 2002; Rohr et al. 2008a, b).

Second, most cases of abnormalities that we found, particularly ectromelia, were detected in agricultural sites, where aquatic environments (except rice systems) are characterized by ephemeral and semipermanent hydroperiod (Peltzer et al. 2006). Generally, ephemeral and temporary bodies of water in this region are free of aquatic insect predators such as Odonata nymphs, diving beetles, and belostomatid bugs (Peltzer and Lajmanovich 2004, 2007). Therefore, predators do not represent a great risk of selective partial or total amputation of hind limbs as demonstrated by Ballengée and Sessions (2009). Although there is evidence that certain predators cause deformities under ecologically relevant conditions (Bowerman et al. 2010; Johnson and Bowerman 2010), more field studies and long-term data are essential to elucidate their exact role in amphibian limb abnormalities.

Third, Ouellet et al. (1997) and Taylor et al. (2005) have suggested increased numbers of major limb abnormalities near agricultural land. In this context, it is important to note that Argentina is a major region of farming of glyphosate-tolerant soybeans, and approximately 200 million liters of glyphosate-based herbicide area applied to produce 50 million tons of soybeans per year (Teubal 2009). The main application time of agrochemicals occurs from November to March, which is coincident with the reproductive period of most amphibians in Argentina (Peltzer and Lajmanovich 2007), as well as with the highest pluvial period. The latter causes intensive pesticide runoff (Peltzer et al. 2008), polluting aquatic ecosystems. Recently, Paganelli et al. (2010) demonstrated how concentrations of glyphosate, lower than those considered relevant environmental concentrations in Argentina wetlands, increased endogenous retinoic acid activity in amphibian embryos and had teratogenic effects. This situation could become even more of a concern with increased teratological risk to amphibians if we consider the effects of certain non-ionic surfactants containing ethoxylates (for example, POEA- polyethoxylates tallawamine) that are added to herbicides. These ingredients are recognized for their influences on normal development on a native tadpole (*Scinax nasicus*) tails, gills, and cephalic structures (Lajmanovich et al. 2003).

Finally, the diversity of possible combinations of potential causes of abnormalities is enormous, which may explain why finding the causes for anuran abnormalities is a difficult task. Hence, it will be necessary for future students to integrate ecological, epidemiological, and developmental tools to solve this environmental mystery (Johnson and Bowerman 2010).

Acknowledgments.—The authors thank Juan Fioramonti for drawings. Especially, we are deeply indebted to Bruce Pauli, David Green, and Michael Lannoo for valuable comments about some abnormalities and essential suggestions that greatly improved early versions of the manuscript. We gratefully acknowledge Christopher Distel for valuable language corrections and helpful remarks. We also thank Eloisa Senkman for providing individuals from a suburban area, Adolfo Beltzer for making available important information on trematodes in aquatic birds and Mariana Cabagna, Walter Cejas, Alfredo Berduc, Rafael F. Lajmanovich Jr. for lab and field assistance. We conducted research under permits provided by Dirección Provincial de Recursos Naturales y Dirección Provincial de Flora y Fauna (Entre Ríos Province), Reserva de Uso Múltiple Parque General San Martín (Entre Ríos Province), Delegación Regional N.E.A., Administración de Parques Nacionales (Entre Ríos Province), Secretaría de Estado de Medio ambiente y Desarrollo Sustentable-Área Fauna (Santa Fe Province), and Agencia Córdoba Ambiente S.E. (Córdoba Province). This research was supported, in part, by PMP and RCL grants or funds from CONICET, SECyT Agency, DAPTF Seed Grant 2005, CAI+D 2005-UNL, and Idea Wildlife Funds 2007.

LITERATURE CITED

Aizen, M.A., L.A. Garibaldi, and M. Dondo. 2009. Expansión de la soja y diversidad de la agricultura Argentina. Ecología Austral 19:45–54.

Ankley, G.T., S.A. Diamond, J.E. Tietge, G.W. Holcombe, K.M. Jensen, D.L. DeFoe, and R. Peterson. 2002. Assessment of the risk of ultraviolet radiation to amphibians. I. Dose-dependent induction of hind limb malformations in the Northern Leopard Frog (*Rana pipiens*). Environmental Science and Technology 36:2853–2858.

Ankley, G.T., J.E. Tietge, G.W. Holcombe, D.L. DeFoe, S.A. Diamond, K.M. Jensen, and S.J. Degitz. 2000. Effects of laboratory ultraviolet radiation and natural sunlight on survival and development of *Rana pipiens*. Canadian Journal of Zoology 78:1092–1100.

Attademo, A.M., P.M. Peltzer, and R.C. Lajmanovich. 2004. Nuevo caso de malformación en un ejemplar de rana (*Leptodactylus ocellatus*) (Amphibia: Anura) del Litoral Argentino. Boletín de la Asociación Herpetológica Española 15:20–22.

Attademo, A.M., P.M. Peltzer, and R.C. Lajmanovich. 2005. Amphibians occurring in Soybean and implications for biological control in Argentina. Agriculture, Ecosystems and Environments 106:389–394.

Ballengée, B., and S.K. Sessions. 2009. Explanation for missing limbs in deformed amphibians. Journal of Experimental Zoology 312:1–10.

Beltzer, A.H., J.A. Schnack, M.A. Quiroga, M.P. Ducommun, A.L. Ronchi Virgolini, and V. Alessio. 2010. Trophic relationships and mechanisms of ecological segregation among heron species in the Parana River floodplain (Birds: Ardeidae). Pp. 49–94 *In* Trends in Ornithological Research. Ulrich, P.K., and J.H. Willett (Eds.). Nova Science Publishers, Hauppauge, New York, USA.

Bowerman, J., P.T.J. Johnson, and T. Bowerman. 2010. Sublethal predators and their injured prey: linking aquatic predators and severe limb abnormalities in amphibians. Ecology 91:242–251.

Burkart, R., N. Bárbaro, R.O. Sánchez, and D.A. Gómez. 1999. Ecorregiones de la Argentina. APN-PRODIA, Buenos Aires, Argentina.

Crump, M.L., and N.J. Scott. 1994. Visual encounter surveys. Pp. 84–91 *In* Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Heyer, W.R., M.A. Donelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster (Eds.). Smithsonian Institution Press, Washington, D.C., USA.

Eaton, B.R., S. Eaves, C. Stevens, A. Puchniak, and C.A. Paszkowski. 2004. Deformity levels in wild populations of the Wood Frog (*Rana sylvatica*) in three ecoregions of Western Canada. Journal of Herpetology 38:283–287.

Fabrezi, M. 1999. Duplicación de la extremidad anterior en *Lepidobatrachus llanensis* (Anura: Leptodactylidae). Cuadernos de Herpetología 13:99–100.

Gardiner, D.M., and D.M. Hoppe. 1999. Environmentally induced limb malformations in Mink Frogs (*Rana septentrionalis*). Journal of Experimental Zoology 284:207–216.

Greenberg, C.H., D.G. Neary, and D. Harris. 1994. A comparison of herpetofaunal sampling effectiveness of pitfall, single ended and double-ended funnel traps used with drift fences. Journal of Herpetology 28:319–324.

Gurushankara, H.P., S.V. Krishnamurthy, and V. Vasudev. 2007. Morphological abnormalities in natural populations of common frogs inhabiting agroecosystems of central Western Ghats. Applied Herpetology 4:39–45.

Hamann, M.I., and C.E. González. 2009. Larval digenetic trematodes in tadpoles of six amphibian species from northeastern Argentina. Journal of Parasitology 95:623–628.

Johnson, P.T.J., and J. Bowerman. 2010. Do predators cause frog deformities? The need for an eco-epidemiological approach. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 314B:515–518.

Johnson, P.T.J., and R.B. Hartson. 2009. All hosts are not equal: explaining differential patterns of malformations in an amphibian community. Journal of Animal Ecology 78:191–201.

Johnson, P.T.J., R.B. Hartson, D.J. Larson, and D.R. Sutherland. 2008. Linking biodiversity loss and disease emergence: amphibian community structure determines parasite transmission and pathology. Ecology Letters 11:1017–1026.

Johnson, P.T.J., K.B. Lunde, E.G. Ritchie, and A.E. Launer. 1999. The effect of trematode infection on amphibian limb development and survivorship. Science 284:802–804.

Johnson, P.T.J., K.B. Lunde, E.G. Ritchie, J.K. Reaser, and A.E. Launer. 2001. Morphological abnormality patterns in a California amphibian community. Herpetologica 57:336–352.

Johnson, P.T.J., K.B. Lunde, E.M. Thurman, E.G. Ritchie, S.N. Wray, D.R. Sutherland, J.M. Kapfer, T.J. Frest, J. Bowerman, and A.R. Blaustein. 2002. Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. Ecological Monographs 72:151–168.

Johnson, P.T.J., D.R. Sutherland, J.M. Kinsella, and K.B. Lunde. 2004. Review of the trematode genus *Ribeiroia* (Psilostomidae): Ecology, life history and pathogenesis with special emphasis on the amphibian malformation problem. Advances in Parasitology 57:191–253.

Kiesecker, J.M. 2002. Synergism between trematode infection and pesticide exposure: A link to amphibian limb deformities in nature? Proceedings of the National Academy of Sciences USA 99:9900–9904.

Labriola, J., and D. Suriano. 1998. Digeneans of birds (Ardeidae) from the Monte Lake, Buenos Aires, Argentina. Physis 56:1–7.

Lajmanovich, R.C. 2000. Interpretación ecológica de una comunidad larvaria de anfibios anuros. Interciencia 25:71–79.

Lajmanovich, R.C., and P.M. Peltzer. 2001. Evaluación de la diversidad de anfibios de un remanente forestal del valle aluvial del río Paraná (Entre Ríos- Argentina). Boletín de la Asociación Herpetológica Española 12:12–17.

Lajmanovich, R.C, J.C. Sánchez-Hernández, P.M. Peltzer, A.M. Attademo, G.S. Fiorenza, M.C. Cabagna, and A. Bassó. 2008. Levels of plasma B-esterase and Glutathione-S-transferase activities in three South American toads. Toxicological and Environmental Chemistry 90:1145–1161.

Lajmanovich, R.C, M.T. Sandoval, and P.M. Peltzer. 2003. Induction of mortality and malformation in *Scinax nasicus* tadpoles exposed by Glyphosate formulations. Bulletin of Environmental Contamination and Toxicology 70:612–618.

Lannoo, M.J. 2008. Malformed Frogs: The Collapse of Aquatic Ecosystems. University of California Press, Berkeley, California, USA.

Lunaschi, L.I., F. Cremonte, and F.B. Drago. 2007. Checklist of digenean parasites of birds from Argentina. Zootaxa 1403:1–36.

Massemin, D., and C. Marty. 2000. A malformed *Dendrobates tinctorius* from French Guiana. Froglog 42:1.

Massone, H.E., D.E. Martinez, J.L. Cionchi, and E. Bocanegra. 1998. Suburban areas in developing countries and their relationships to groundwater pollution: A case study of Mar del Pata, Argentina. Environmental Management Journal 22:245–254.

Meteyer, C.U. 2000. Field guide to malformations of frogs and toads with radiographic interpretations. U.S. Geological Survey Biological Science Report USGS/BRD/BSR-2000-0005.

Ostrowski de Nuñez, M. 1968. Estudios sobre la fauna parasitaria del biguá, *Phalacrocorax o. olivaceous.* Trematodes pertenecientes a las familias Cathaemasidae y Echinostomatidae. Revista del Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Parasitología 1:31–152.

Ouellet, M. 2000. Amphibian deformities: Current state of knowledge. Pp. 617–661 *In* Ecotoxicology of Amphibians and Reptiles. Sparling, D.W., G. Linder, and C.A. Bishop (Eds.). Society of Environmental Toxicology and Chemistry (SETAC) Press, Pensacola, Florida, USA.

Ouellet, M., J. Bonin, J. Rodrigue, J. Desgranges, and S. Lair. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. Journal of Wildlife Diseases 33:95–104.

Paganelli, A, V. Gnazzo, H. Acosta, S.L. López, and A.E. Carrasco. 2010. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chemical Research in Toxicology 23:1586–1595.

Peltzer, P.M., and R.C. Lajmanovich. 2004. Anuran tadpole assemblages in riparian areas of Paraná River (Argentina). Biodiversity and Conservation 13:1833–1842.

Peltzer, P.M., and Lajmanovich, R.C. 2007. Amphibians. Pp. 327–340 *In* The Middle Paraná River: Limnology of a Subtropical Wetland. Iriondo, M.H., J.C. Paggi, and M.J. Parma (Eds.). Springer Publishing, New York, New York, USA.

Peltzer, P.M., R.C. Lajmanovich, A.M. Attademo, and A.H. Beltzer. 2006. Diversity of anurans across agricultural ponds in Argentina. Biodiversity and Conservation 15:3499–3513.

Peltzer, P.M., R.C. Lajmanovich, and A.H. Beltzer. 2003. The effects of habitat fragmentation on amphibian species richness in the floodplain of the middle Paraná River. Herpetological Journal 13:95–98.

Peltzer, P.M., R.C. Lajmanovich, J.C. Sánchez-Hernández, M. Cabagna, A.M. Attademo, and A. Bassó. 2008. Effects of agricultural pond eutrophication on survival and health status of *Scinax nasicus* tadpoles. Ecotoxicology and Environmental Safety 70:185–197.

Peltzer, P.M., M.L. Ponzza, and R.C. Lajmanovich. 2001. Caso de malformación en *Leptodactylus mystacinus* (Anura: Leptodactylidae). Natura Neotropicalis 32:173–176.

Peri, S., and J. Williams. 1988. Anomalías osteológicas en *Hyla pulchella pulchella* y *Pseudis paradoxus platensis* (Amphibia: Anura). Boletín de la Asociación Herpetológica Argentina 4:4–5.

Piha, H., M. Pekkonen, and J. Merila. 2006. Morphological abnormalities in amphibians in agricultural habitats: A case study of the Common Frog *Rana temporaria*. Copeia 2006:810–817.

Prigione, C.M., and J.A. Langone. 1985. Anomalías anatómicas registradas en anfibios de la colección herpetológica del Museo Nacional de Historia Natural. Actas de las Jornadas de Zoología del Uruguay 1:73–75.

Read, J.L., and M.J. Tyler. 1990. The nature and incidence of post-axial, skeletal abnormalities in the frog *Neobatrachus centralis* Parker at Olympic Dam, South Australia. Transactions of the Royal Society of South Australia 114:213–217.

Rohr, J.R., T.R. Raffle, S.K. Sessions, and P.J. Hudson. 2008a. Understanding the net effects of pesticides on amphibian trematode infections. Ecological Applications 18:1743–1753.

Rohr, J.R., A.M. Schotthoefer, T.R. Raffel, H.J. Carrick, N. Halstead, J.T. Hoverman, C.M. Johnson, L.B. Johnson, C. Lieske, M.D. Piwoni, P.K. Schoff, and V.R. Beasley. 2008b. Agrochemicals increase trematode infections in a declining amphibian species. Nature 455:1235–1239.

Sessions, S.K., and S.B. Ruth. 1990. Explanation for naturally occurring supernumerary limbs in amphibians. Journal of Experimental Zoology 254:38–47.

Skelly, D.K., S.R. Bolden, L.K. Freidenburg, N.A. Freidenfelds, and R. Levey. 2007. *Ribeiroia* infection is not responsible for Vermont amphibian deformities. EcoHealth 4:156–163.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry: The Principles and Practices of Statistics in Biological Research. 3rd Edition. W.H. Freeman and Company, New York, New York, USA.

Takeishi, M. 1996. On the frog, *Rana ornativentris*, with supernumerary limbs found at Yamada Green Area in Kitakyushu City, Fukuoka Prefecture, Japan. Bulletin of the Kitakyushu Museum of Natural History 15:119–131.

Taylor, B., D. Skelly, L.K. Demarchis, M.D. Slade, D. Galusha, and P.M. Rabinowitz. 2005. Proximity to pollution sources and risk of amphibian limb malformation. Environmental Health Perspectives 113:1497–1501.

Teubal, M. 2009. Expansión del modelo sojero en la Argentina. De la producción de alimentos a los commodities. Pp. 161–197. *In* La Persistencia del Campesinado en América Latina. Lizárraga, P., and C. Vacaflores (Eds.). Comunidad de Estudios JAINA, Tarija, Bolivia.

Vandenlangenberg, S.M., J.T. Canfield, and J.A. Magner. 2003. A regional survey of malformed frogs in Minnesota (USA) (Minnesota malformed frogs). Environmental Monitoring and Assessment 82:45–61.

Voitkevich, A.A. 1961. Le développement des extrémités surnuméraires chez les Amphibiens. Bulletin biologique de la France et de la Belgique 95:569–600.

Zimmerman, B.L. 1994. Audio strip transects. Pp. 92–97 *In* Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Heyer, W.R., M.A. Donelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster (Eds.). Smithsonian Institution Press, Washington, D.C., USA.

Appendix

Institutions where specimens were deposited:

MFV: Natural Sciences Museum "Florentino Ameghino", Santa Fe Province
DCN-UNRC: Ecology signature, Natural Sciences Department, Faculty of Mathematics, Physics and Chemistry and Natural, National University of Río Cuarto, Córdoba Province
DIAM: Center for Scientific Research and Technology Transfer to Production, Diamante, Entre Rios Province
FBCB: Ecotoxicology Laboratory, Faculty of Biochemistry and Biological Sciences, National University of Litoral, Santa Fe Province

(Left to Right) Junges, Attademo, Lajmanovich, Peltzer, and Sanchez. (Photographed by Agustín Bassó)

Paola Mariela Peltzer holds a Licenciate in Biodiversity (National University of Litoral-UNL), Argentina and a Doctorate in Natural Sciences (Zoology; La Plata University-UNLP). She is a Senior Investigator of the National Council for Scientific and Technical Research (CONICET) in the Ecotoxicology Laboratory (ESS-FBCB-UNL). She has expertise in the ecology and conservation of anurans in altered environments, particularly in ecological responses related to habitat fragmentation and in agroecosystems. She is a teacher in the UNL. Blog: http://anfibios-ecotox-conser.blogspot.es/.

Rafael Carlos Lajmanovich has a Licenciate in Biodiversity (National University of Litoral-UNL), Argentina, and a Doctorate in Natural Sciences (Zoology; La Plata University-UNLP). He is a Senior Investigator of the National Council for Scientific and Technical Research (CONICET). He is the director of the Ecotoxicology Laboratory (ESS-FBCB-UNL). His experience includes ecological and biochemical responses of anurans in modified environments, particularly in agroecosystems. He is a Titular teacher at the UNL.

Laura Cecilia Sanchez has a Licenciate in Biological Sciences, (Buenos Aires University-UBA). She has a Doctorate in Biological Sciences from the National University of Litoral (UNL), with the support of National Council for Scientific and Technical Research (CONICET). She is an auxiliary teacher at the Entre Ríos National University (UNER).

Andrés Maximiliano Attademo has a Licenciate in Biological Sciences (Córdoba University-UNC) and a Doctorate in Biological Sciences from the National University of Litoral (UNL), with the support of National Council for Scientific and Technical Research (CONICET). He works in the Ecotoxicology Laboratory (ESS-FBCB-UNL). He is a practical lab teacher at the UNL. He has expertise in the biochemical responses (enzyme activities) of anurans in agroecosystems.

Celina María Junges has a Licenciate in Environmental Reparation (National University of Litoral-UNL). She is a doctoral student in Biological Sciences at the UNL, with the support of the National Council for Scientific and Technical Research (CONICET). She works in the Ecotoxicology Lab (ESS-FBCB-UNL). She is a practical lab teacher at the UNL. She has expertise in predator-prey interactions of anurans in agroecosystems and in the ecological responses of anurans in simulated polluted ponds.

Clarisa Lourdes Bionda has a Licenciate in Biology (National University of Río Cuarto-UNRC). She has a Doctorate in Biological Sciences from the UNRC, with the support of National Council for Scientific and Technical Research (CONICET). She has expertise in the ecology of anurans in agroecosystems in Central Argentina. (Photographed by Adolfo Martino)

Adolfo Martino has a Licenciate in Biology (National University of Río Cuarto-UNRC), Argentina and a Doctorate in Biological Sciences (UNRC). He is a Senior Investigator and works in the ecology section within the Department of Natural Science (UNRC). He is a Titular teacher at the UNRC. His experience is in bioecology and in vocalization of anurans in Central Argentina. (Photographed by Rafael Lajmanovich Jr.)

Agustín Bassó is an advanced student working on a Licenciate in Biodiversity at the National University of Litoral -UNL, Argentina. He is a practical lab teacher at the UNL. He has expertise in the ecology of anurans in natural and altered environments in the mid-eastern of Argentina. (Photographed by Paola Peltzer)