The Herpetological Bulletin 160, 2022: 27–30
SHORT COMMUNICATION

https://doi.org/10.33256/hb160.2730

Reproductive behaviour of the threatened rusted frog *Telmatobius rubigo*

MARIA S. GASTÓN

Instituto de Ecorregiones Andinas, Universidad Nacional de Jujuy, Consejo Nacional de Investigaciones Científicas y Tecnológicas, Canónigo Gorriti 237, 4600 San Salvador de Jujuy, Argentina

Author e-mail: solegaston@gmail.com

The genus *Telmatobius* Wiegmann, 1834 (Anura: Telmatobiidae) includes 63 species of aquatic and semi-aquatic frogs associated with high Andean ecosystems and distributed from Ecuador to Chile and Argentina (Frost, 2021). The high-altitude distribution and the aquatic habits of *Telmatobius* are its most distinctive characteristics and pose a series of physiological challenges for the genus (Lavilla & De la Riva, 2005; Barrionuevo, 2017). *Telmatobius* frogs were used by the Inca culture as symbols to propitiate rainfall and fertility (Elías et al., 2019; Otero et al., 2020), possibly due to frogs' conspicuous reproductive biology (Wells, 2007). Despite this, there is little information about the reproduction of these Andean water frogs. The genus appears to have low fecundity (Barrionuevo & Mangione, 2006), with clutch size varying from 80 eggs (Pisanó, 1955) to about 500 eggs per clutch for *Telmatobius culeus* and *Telmatobius laticeps* (Pérez, 1998; Barrionuevo & Mangione, 2006). Some earlier evidence suggests that male game togenesis in *T. laticeps* and *Telmatobius pisanoi* occurs twice yearly (Montero & Pisanó, 1990) but is continuous and asynchronous in females of *Telmatobius arequipensis* (reviewed Lavilla & Barrionuevo, 2005). *T. culeus* probably reproduces continuously as amplexus was recorded mainly during winter months and females with mature ovules were recorded in the summer (Pérez, 1998). *Telmatobius jelskii* probably also reproduces throughout the year as eggs, breeding adults, and cohorts of tadpoles at several developmental stages have been observed in both wet and dry seasons (Catenazzi et al., 2013).

In Argentina, the 15 species of the genus are threatened (Vaira et al., 2017) due to habitat alteration, the introduction of exotic predatory fishes, chytrid fungus infection and the indirect consequences of extreme climate events (Barrionuevo & Mangione, 2006; Barrionuevo & Ponssa, 2008; Vaira et al., 2012; IUCN, 2021). However, there is limited knowledge of the natural history of Andean water frogs (Lavilla & Barrionuevo, 2005; Barrionuevo & Abdala, 2018; Acosta et al., 2020; Araos et al., 2022). The Rusted Frog *Telmatobius rubigo* Barrionuevo & Baldo 2009 is a fully aquatic frog endemic to the endorheic basin of the Laguna de Los Pozuelos in the Central Andean Puna ecoregion of Jujuy province in Argentina (Barrionuevo & Baldo, 2009), at 3,500 - 4,300 m a.s.l. This frog has a unique feeding mechanism among Neobatrachia, using inertial suction to capture their prey (Barrionuevo, 2016), mainly aquatic coleopterans, insects, and crustaceans (Akmentins & Gastón, 2020). It appears that the larval period in *T. rubigo* lasts more than a year, given the simultaneous occurrence of different larval stages in the same site and the low temperatures of the high mountain permanent streams inhabited by the species (Barrionuevo, 2018). Other aspects of their natural history, such as breeding behaviour and reproductive ecology, have not been reported. Here, I present novel data on the reproductive behaviour of *T. rubigo* related to amplexus, clutch characteristics, comments on developmental stages of embryos, and the reproductive mode of the species.

I carried out frog surveys in 2018, 2020 and 2021 at Laguna de Los Pozuelos basin and the surrounding area in the Central Andean Puna ecoregion, north-west Argentina. Here, the altitude and widening of the Andes produce specific climatic conditions being a semi-arid region, with high-temperature fluctuations, intense solar radiation, and high evaporation (Murray et al., 2019). Data from the meteorological station in Natural Monument Laguna de Los Pozuelos (22° 28'24.00" S, 65° 59'35.00" W; 3,675 m a.s.l) indicates an annual mean air temperature of 8 °C (range -19° to 27 °C), annual mean relative air humidity of 44 % and annual mean precipitation of 317 mm for the 2018 and 2019 period (data provided by Administración de Parques Nacionales of Argentina). Precipitation occurs mainly in the austral summer between December and March (wet season), and the hydric deficit increases from April to November (dry season) (Murray et al., 2019). The watercourses of the Pozuelo's basin are typical of the high-altitude grassland being mostly alkaline (pH values > 7) and highly oxygenated (dissolved oxygen generally range from 8-10.6 mg/L), the conductivity increases from the headwaters of the tributary rivers to the centre of the basin with values from 140 to 448 µS/cm (Murray et al., 2019).

On 23 January 2018, I observed one axillary amplexus of *T. rubigo*, with male clasping female behind the front legs (Fig. 1A), at Cusi Cusi locality (22° 22'8.9" S, 66° 13'29.7" W; 4333 m a.s.l.). A total of ten egg clutches of *T. rubigo* were recorded in three localities, five clutches in Austral summer (wet season) and five clutches in Austral winter (dry season) (Table 1). The sites of the clutches were wells or backwater in the beds of permanent streams (Fig. 1 B–D). Across both seasons in Santa Catalina, five clutch sites were measured with a wooden ruler and the mean (±sd) depth and width

were 10.27 ± 3.15 cm and 179 ± 64.65 cm respectively. All eggs were included in a gelatinous mass attached to the underside of flat rocks and some subaquatic vegetation in permanent streams (Fig. 2 A & B), in agreement with previous descriptions of *Telmatobius* spp. in Argentina (Lavilla & Barrionuevo, 2005). Nine clutches were photographed next to a photo scale using a digital camera, and images were processed using ImageJ 1.53K software to estimate the clutch and egg sizes.These clutches had between 34 and 390 eggs. The eggs showed a black pigmented animal pole and a white vegetative pole (Fig. 1 B & C) and had a mean (±sd) diameter of 0.17 ± 0.02 cm (range 0.11–0.26 cm), similar to egg sizes mentioned for *T. culeus* (Pérez, 1998) and *T. hauthali* (reviewed in Lavilla & Barrionuevo, 2005). Some embryological characteristics of *T. rubigo* were observed and are described according to the terminology of Gilbert (2000) and Gosner (1960). The grey crescent is present in fertilised eggs, the small, pigmented, furrowed animal pole and the large vegetative pole were observed in cleaved eggs, the yolk plug and blastopore lips indicate the gastrulation, the neural plate and neural tube formation are present at neurulation, and protuberances of the forebrain, tail bud, muscular responses, and body pigmentation were present in the pre-hatching tadpole (Fig.2 A–F). In some cases, these embryological stages were present in the same egg clutch (Fig. 2 D & F).

In Santa Catalina, four clutches associated with different males of *T. rubigo* (one male per clutch) where observed in March 2021 and September 2021. Each male was resting under the rock that had the clutch. Adult frogs and tadpoles at all development stages were observed in all surveys, as previously reported by Barrionuevo (2018).

The present observations suggest that *T. rubigo* breeds during the wet and dry seasons, laying eggs in clutches in permanent shallow and highly oxygenated waters. Since eggs, adults of both sexes and different stages of tadpole development were observed in both wet and dry seasons, it is suggested that *T. rubigo* reproduces continuously. According to the reproductive mode (RM) classification of Nunes-de-Almeida et al. (2021) the species seems to conform to RM 16, in that it lays eggs on the bottom of fast moving fresh water and has free-living tadpoles that feed on nutrients in their environment without parental support (i.e. do not rely on trophic eggs). Axillary amplexus has been observed in *T. culeus* and has been suggested typical of the *Telmatobiidae* (Mantilla Mendoza, 2018; Carvajal-Castro et al., 2020) and is now confirmed for *T. rubigo*. For *T. culeus*, the amplexus lasts up to three days with lengthy oviposition in bouts

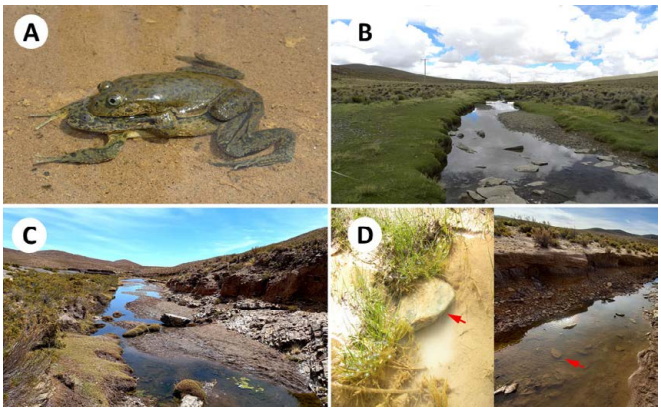

Figure 1. *Telmatobius rubigo* and its habitats in Argentina- **A.** A pair in axillary amplexus, **B.** & **C.** Permanent streams at Casa Colorada and Santa Catalina localities where egg clutches and individual *T. rubigo* were observed, **D.** Clutch sites of *T. rubigo* (arrows indicate groundwater rocks where the clutches were found)

Table 1. Egg clutches of *Telmatobius rubigo* observed during frog surveys in the Central Andean Puna ecoregion, Argentina

Locality	Co-ordinates	Date	Seasons	N° of clutches	N° of eggs	Developmental stage
Casa Colorada	22° 22'8.9" S, 66° 13'29.7" W 4,333 m a.s.l.	23 Jan 2018	Wet/Summer	1	140*	C, P
		12 Jan 2020	Wet/Summer	1	np	np
Cusi Cusi	22° 24'15.7" S, 66° 32'25.4" W 3,803 m a.s.l.	24 Jan 2018	Wet/Summer	1	88	F
Santa Catalina	21° 56'58.2" S, 66° 02'21.6" W 3,802 m a.s.l.	9 March 2021	Wet/Summer	2	349	F, C, N
					159	F
		24 August 2021	Dry/Winter	1	295	G
		13 Sept 2021	Dry/Winter	4	63	F, C, N, P
					390	P
					34	C
					296	C, P

Abbreviations: C- cleavage; F- fertilisation; G- gastrulation; N- neurulation; np-, not photographed; P- pre-hatching. *Minimum number counted because there were dirt sediments in the gelatinous mass.

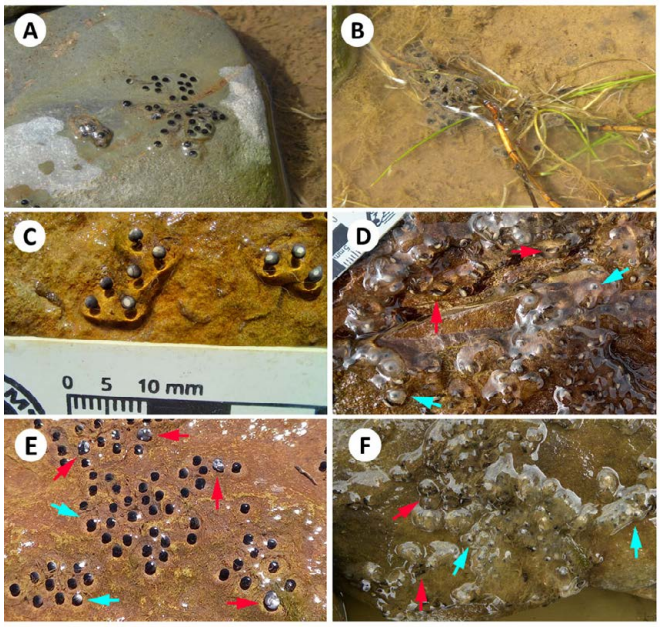

Figure 2. Clutch characteristics of *T. rubigo* - **A.** & **B.** A clutch of eggs attached to the underside of a rock and subaquatic vegetation. The eggs are in the fertilisation stage by the appearance of the grey crescent. **C.** Eggs in gastrulation, visualising yolk plug and blastopore lip. **D.**, **E.** & **F.** Clutches with different development stages of the embryo. Light blue arrows indicate eggs or embryos in the early stages of development (fertilisation-gastrulation), red arrows indicate embryos at more advanced development stages (neurulation -pre-hatching).

releasing a small proportion of the eggs throughout these days (Ramos, 2000; Pérez, 2005; Mantilla Mendoza, 2018). *T. rubigo* appears to have a similar oviposition behaviour. The difference in clutch sizes reported for the species suggests consecutive laying events of small egg masses (~ 30 eggs) that could eventually result in an egg mass of ~ 400 eggs. If spawning occurs at the same place, then embryos at different development stages would be found in a clutch consistent with the data presented here for *T. rubigo*. Nevertheless, the possibility that there are instead community clutches, as suggested for other species (Mantilla Mendoza, 2018), cannot be dismissed. Further studies are needed to unveil this behaviour and other reproductive aspects of *T. rubigo*, such as parental care behaviour that has been suggested by the association of males with the clutches.

T. rubigo is threatened by direct and indirect consequences of human activities (Vaira et al., 2012; IUCN, 2021). To the threats observed during fieldwork by Akmentins & Gastón (2020) I would now add climate change. Increases in extreme precipitation events were reported for the region (Alabar et al., 2020) and occurred in Santa Catalina during the weeks before my 2021 fieldwork. An intense hailstorm and a historic stream flood during February and March of 2021 damaged crops and livestock (local news and residents comm.) and modified the stream's banks, removing stones and vegetation of frog shelters (pers. obs.). Unusual climate coupled with an increase in erosive processes and debris flowing events in mountain streams were associated with population declines of other *Telmatobius* from Argentina (Barrionuevo & Ponssa, 2008). Thus, the effect that increasingly frequent extreme climatic events have on the biology of the threatened frog *T. rubigo* needs to be thoroughly studied. Knowing how reproductive behaviour can be affected by climate change and other human-related threats is crucial for developing conservation strategies for the species.

ACKNOWLEDGEMENTS

I want to thank Teresa, Fortunata, and the local people of Santa Catalina town for sharing their experiences with climate events. I thank M.S. Akmentins and M. Vaira for fieldwork assistance and volunteering revision of the manuscript. I thank J.H. Urquizo for his fieldwork assistance. Partial funding was provided by PUE INECOA 22920170100027CO and PICT 2019-4177. Permits for fieldwork were provided by Ministerio de Ambiente, Dirección Provincial de Biodiversidad de Jujuy, Argentina (171/2015 DPB).

REFERENCES

Acosta, R., Vera, R., Castro Cavicchini, S., Núñez, A., González Turu, N., AbdenurAraos, F. & Figueroa, R. (2020). Aspectosecológicos de *Telmatobius atacamensis* (Anura: Telmatobiidae), un microendemismo de la Puna, Salta–Argentina. *Revista peruana de biología* 27: 113–120.

Akmentins, M.S. & Gastón, M.S. (2020). Feeding habits of the threatened aquatic Andean frog *Telmatobius rubigo* (Anura: Telmatobiidae). *Amphibian & Reptile Conservation* 14: 162–168.

Alabar, F., Hurtado, R., Corte, M.V. & Moreno, C. (2020). Análisis temporal de índices climáticos de precipitación de las provincias de Jujuy y Salta. *Revista Científica de la Facultad de Ciencias Agrarias -UNJu* 13: 7–13.

Araos, F.A., Vera, R., Castro-Cavicchini, S., González-Turu, N., Núñez, A. & Acosta, R. (2022). Dieta de *Telmatobius atacamensis* (Anura: Telmatobiidae): primeros aportes al conocimiento sistemático del nicho trófico. *Caldasia* 44(3).

Barrionuevo, J.S. (2016). Independent evolution of suction feeding in Neobatrachia: feeding mechanisms in two species of *Telmatobius* (Anura: Telmatobiidae). *The Anatomical Record* 299: 181–196.

Barrionuevo, J.S. (2017). Frogs at the summits: phylogeny of the Andean frogs of the genus *Telmatobius* (Anura, Telmatobiidae) based on phenotypic characters. *Cladistics* 33: 41–68.

Barrionuevo, J.S. (2018). Growth and cranial development in the Andean frogs of the genus *Telmatobius* (Anura: Telmatobiidae): Exploring the relation of heterochrony and skeletal diversity. *Journal of Morphology* 279: 1269–1281.

Barrionuevo, J.S. & Abdala, C.S. (2018). Herpetofauna de la Puna Argentina: una síntesis. In *La Puna Argentina: Naturaleza y Cultura*. Serie Conservación de la Naturaleza 24, 209–228 pp. Grau, H.R., Babot, M.J., Izquierdo, A.E. & Grau, A. (Eds.) Fundación Miguel Lillo, Tucumán, Argentina.

Barrionuevo, J.S. & Baldo, D. (2009). A new species of

Telmatobius (Anura, Ceratophryidae) from northern Jujuy Province, Argentina. *Zootaxa* 2030: 1–20.

Barrionuevo, J.S. & Mangione, S. (2006). Chytridiomycosis in two species of *Telmatobius* (Anura; Leptodactylidae) from Argentina. *Diseases of Aquatic Organisms* 73: 171–174.

Barrionuevo, J.S. & Ponssa, M.L. (2008). Decline of three species of the genus *Telmatobius* (Anura: Leptodactylidae) from Tucumán Province, Argentina. *Herpetologica* 64: 47–62.

Carvajal-Castro, J.D., López-Aguirre, Y., Ospina-L, A.M., Santos, J.C., Rojas, B. & Vargas–Salinas, F. (2020). Much more than a clasp: evolutionary patterns of amplexus diversity in anurans. *Biological Journal of the Linnean Society* 129: 652–663.

Catenazzi, A., von May, R. & Vredenburg, V.T. (2013). Conservation of the high Andean frog *Telmatobius jelskii* along the PERU LNG pipeline in the Regions of Ayacucho and Huancavelica, Peru. In *Monitoring biodiversity: Lessons from a Trans–Andean megaproject*. Alonso, A., Dallmeier, F. & Servat, G. (Eds.). Smithsonian Scholarly Press, Washington DC. 26 pp.

Elías, R., Ramos, E., García, J., Herbert, M. & Quispe, J. (2019). Calling the Rains With the Help of the Lake Titicaca Frog. *FrogLog* Issue 121: 27(1). 22.

Frost, D.R. (2021). Amphibian Species of the World: an Online Reference. Version 6.1 (Date of access). Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA.

Gilbert, S.F. (2000). *The Frog Life Cycle in Developmental Biology*. 6th edition. Sunderland (MA): Sinauer Associates. https://www.ncbi.nlm.nih.gov/books/NBK10035/

Lavilla, E.O. & Barrionuevo, J.S. (2005). El género *Telmatobius* en la República Argentina: una síntesis. *Monografías de Herpetología* 7: 115–165.

Lavilla, E.O. & De la Riva, I. (2005). Estudios sobre las ranas andinas de los géneros *Telmatobius* y *Batrachophrynus* (Anura: Leptodactylidae). *Monografías de Herpetología* 7: 65–101.

Mantilla Mendoza B. (2018). Reproducción de la Rana Gigante (*Telmatobius culeus*, Garman 1875) del Lago Titicaca en ambientes controlados, PUNO. Tesis. Universidad Nacional del Altiplano. 91 pp.

Montero, R. &. Pisanó A. (1992). Ciclo anual de lavitelogénesis en tres especies de anuros del noroesteargentino. *Alytes* 9:103–119.

Murray, J., Nordstrom, D.K., Dold, B., Orué, M.R. & Kirschbaum, A. (2019). Origin and geochemistry of arsenic in surface and groundwaters of Los Pozuelos basin, Puna region, Central Andes, Argentina. *Science of the Total Environment* 697: 134085.

Nunes–de–Almeida, C.H.L., Haddad, C.F.B. & Toledo, L.F. (2021). A revised classification of the amphibian reproductive modes. *Salamandra* 57: 413–427.

Otero, C., Akmentins, M.S. & Quinteros, A.S. (2021). Animales en acción: usos rituales de fauna silvestre y de representaciones zoomorfas en contextos incaicos del Pucará de Tilcara (Quebrada de Humahuaca, Argentina). *Estudios atacameños* 67: e3926.

Pérez, M.E. (1998): Dieta y Ciclo Gametogénico Anual de *Telmatobius culeus* (Anura: Leptodactylidae) en el Lago Titicaca (Huiñaimarca). *Tesis de Licenciatura en Biología*, Universidad Mayor de San Andrés, La Paz, Bolivia.

Pérez, M.E. (2005). Cría en cautividad y uso sostenible de la rana gigante del lago Titicaca (*Telmatobius culeus*). *Monografías de Herpetología* 7: 261–271.

Pisanó, A. (1955) Notas ecológicas sobre la vida larvaria de *Telmatobius schreiteri*. *Ciencia e Investigación* 11: 86–91.

Ramos, L. Gallegos, N. & Sulma, L. (2000). Evaluación de la información disponible de suri, pisaca y rana gigante del lago. Universidad Nacional del Altiplano de Puno. Facultad de Ciencias Biológicas. 109 pp.

Vaira, M., Akmentins, M., Attademo, M., Baldo, D., Barrasso, D., Barrionuevo, S., Basso, N., Blotto, B., Cairo, S., Cajade, R. et al. (2012). Categorización del estado de conservación de los anfíbios de La República Argentina. *Cuadernos de Herpetología* 26: 131–159.

Vaira, M., Pereyra, L.C., Akmentins, M.S. & Bielby, J. (2017). Conservation status of amphibians of Argentina: An update and evaluation of national assessments. *Amphibian & Reptile Conservation* 11: 36–44 (e135).

Wells, K.D. (2007). *The Ecology and Behavior of Amphibians*. University of Chicago Press. 1148 pp.

Accepted: 10 December 2021