A field method for sampling blood of male anurans with hypertrophied limbs

Article · June 2011

DOI: 10.11606/issn.2316-9079.v10i1p69-73

3 authors:

Benjamin Tapley
ZSL
79 PUBLICATIONS **403** CITATIONS
SEE PROFILE

Andrés Rymel Acosta-Galvis
Instituto de Investigación de Recursos Biológicos Alexander von Humboldt
95 PUBLICATIONS **501** CITATIONS
SEE PROFILE

Javier Lopez
Chester Zoo
41 PUBLICATIONS **156** CITATIONS
SEE PROFILE

Phyllomedusa 10(1):75–77, 2011

ISSN 1519-1397

SHORT COMMUNICATION

A field method for sampling blood of male anurans with hypertrophied limbs

Benjamin Tapley[1], Andrés Rymel Acosta-Galvis[2], and Javier Lopez[1]

[1] Durrell Wildlife Conservation Trust, Les Augrès Manor, La Profonde Rue, Trinity, Jersey, British Channel Islands, JE3 5BP. E-mail: ben_tapley@hotmail.com.

[2] Calle 174, 8-31 CS 77, Bogotá D.C., Colombia.

Keywords: Anura, blood sampling, haematology, minimally invasive.

Palavras-chave: Anura, amostra de sangue, hematologia, procedimento minimamente invasivo.

Blood analysis is an essential tool in evaluating the health of amphibians; examination and analysis of blood cells provide important information on blood-parasite levels (Desser 2001), the status of different organ systems, and insights on the status of the immune system (Marnila *et al*. 1995). Blood samples can also be analyzed for genetic, toxicological, and stable isotopes and for disease in general or for the presence of specific infectious diseases (Bulté *et al*. 2006). Challenges of amphibian venipuncture include the small sizes of specimens with few available venipuncture sites (Heatley and Johnson 2009). Non-invasive or minimally invasive sampling methods are preferable when working with endangered or declining amphibian species (Pidancier *et al*. 2003).

Blood samples are usually obtained from the lingual venous plexus (Baranowski-Smith and Smith 1983), midline abdominal vein (Wright and Whitaker 2001), femoral vein (Hadfield and Whiticker 2005) or from the heart (Wright and Whitaker 2001) in anurans. Because the small size of vessels can make collection of blood difficult, cardicentesis usually is used to sample smaller species. However this technique requires anaesthesia to reduce the risks of cardiac lacerations and has been associated with cardiac arrest (Hadfield and Whiticker 2005). Blood samples also can be taken when toes are clipped (Wright and Whitaker 2001); however, this method is invasive. Given the existence of other blood sampling methods, obtaining blood samples from toe clipping is not recommended unless the toe clipping is part of a wider study in which toe clipping is being used for identification purposes. It is recommended to limit the maximum volume of the blood sample to less than 1% of the body weight of a healthy amphibian (Wright and Whitaker 2001).

While conducting a routine herpetological survey in Albania, Municipality of Mariquita, Colombia (Departmento de Tolima) in November 2008, we collected three adult male *Leptodactylus bolivianus* (Boulenger, 1898) in breeding condition (Figure 1A). During the breeding season, male *L. bolivianus* develop hypertrophied arms. These are medium-sized, territorial frogs (male

Received 14 April 2011.
Accepted 21 June 2011.
Distributed July 2011.

snout–vent length 90 mm), (Sexton 1962). It is likely that the forelimb muscular hypertrophy is associated with male combat (Wells 2007) and breeding seasonality.

There are large cephalic veins associated with the hypertrophied arms of *Leptodactylus bolivianus*. Blood was taken from the cephalic veins of three individuals with a 0.5-ml insulin syringe (Figure 1B). The needle was angled at 45° and inserted into the vein. The plunger from the syringe was withdrawn to the 0.1-ml mark to create a vacuum. The syringe was rotated and/or its angle of insertion slowly increased or decreased until blood was withdrawn. Because this procedure was carried out as a test of a concept rather than part of a blood study, we withdrew the needle when 0.05 ml of blood was collected. Blood smears were prepared from the blood samples. The individuals sampled did not exhibit haematoma at the site of venepuncture after 30 min. These frogs were euthanized with tricaine methanesulfonate (MS222) and deposited as voucher specimens in the amphibian collections of Pontificia Universidad Javeriana, Bogotá. The blood smears showed no abnormalities or parasites and are archived at the veterinary department at Durrell Wildlife Conservation Trust. The Declining Amphibian Task Force code of practice for amphibian related fieldwork was followed. Powder free latex gloves were worn at the collection site and when sampling blood from the frogs.

Taking blood samples from the cephalic vein is minimally invasive and does not require chemical restraint. There are no major underlying organs, minimizing the risks of severe trauma were the frog to move suddenly during sample collection. (This is a potential risk in using non peripheral vernipuncture sites.) This technique probably is limited to sampling male anurans with hypertrophied arms, including, but not limited to, some species of the following families: Bufonidae (*Bufo* and *Rhinella*), Dicroglossidae (*Quasipaa*), Hylidae (*Bokermannohyla*, *Hyloscirtus*, *Hypsiboas* and *Plectrohyla*), Leptodactylidae (*Leptodactylus*), Limnodynastidae (*Limnodynastes*), Megophryidae (*Leptobrachium*), Petropedetidae (*Petropedetes*), and Ranidae (*Babina*, *Lithobates* and *Rana*) (Oka *et al*. 1984, Ho *et al*. 1999, Peters and Aulner 2000, Navas and James 2007, Wells 2007). We consider this blood-sampling technique to be useful because male anurans frequently are encountered on routine herpetological surveys in breeding congregations.

Figure 1. (**A**) Adult male *Leptodactylus bolivianus* in breeding condition. Note hypertrophied arms. (**B**) Drawing blood from the cephalic vein.

Acknowledgments.—Fieldwork was made possible with the cooperation of Monica Donuyer of CORPOCALDAS. Special thanks to the Pangea Foundation, Mauricio Duran who accompanied us during all the fieldwork, and all the people of the village of Albania for their hospitality. Thanks to Gerardo Garcia and Eluned Price for their comments on the original manuscript. Finally we thank The Durrell Wildlife Conservation Trust and Robin Rumboll for their financial support.

References

Baranowski-Smith, L. L. and C. J. V. Smith. 1983. A simple method for obtaining blood samples from mature frogs. *Laboratory Animal Science 33:* 386–387.

Bulté, G., C. Verly, and G. Blouin-Demers. 2006. An improved blood sampling technique for hatchling emydid turtles. *Herpetological Review 37:* 318–319.

Desser, S. S. 2001. The blood parasites of anurans from Costa Rica with reflections on the taxonomy of their trypanosomes. *Journal of Parasitology 87:* 152–160.

Hadfield, C. A. and B. R. Whiticker. 2005. Amphibian emergency medicine and care. *Seminars in Avian and Exotic Medicine 14:* 79–89.

Heatley, J. J. and M. Johnson. 2009. Clinical Technique: Amphibian Haematology: A Practitioner's guide. *Journal of Exotic Pet Medicine 18:* 14–19.

Ho, C. T., A. Lathrop, R. W. Murphy, and N. Orlov. 1999. A redescription of *Vibrissaphora ailaonica* with a new record in Vietnam. *Russian Journal of Herpetology 6:* 48–54.

Marnila, P., A. Tiiska, K. Lagerspetz, and E. M. Lilius. 1995. Phagocyte activity in the frog *Rana temporaria:* whole blood chemiluminescence method and the effects of temperature and thermal acclimation. *Comparative Biochemistry and Physiology. Part A: Physiology 111:* 609–614.

Navas, C. A and R. S. James. 2007. Sexual dimorphism of exterior carpi radialis muscle size, isometric force, relaxation rate and stamina during the breeding season of the frog *Rana temporaria* Linnaeus 1758. *The Journal of Experimental Biology 210:* 715–721.

Oka, Y., R. Ohtani, M. Satou, and Ueda, K. 1984. Sexually dimorphic muscles in the forelimb of the Japanese toad, *Bufo japonicus*. *Journal of Morphology 180:* 297–308.

Peters, S. E. and D. A. Aulner. 2000. Sexual dimorphism in the forelimb muscles of the bullfrog, *Rana catesbeiana:* a functional analysis of isometric contractile properties. *Journal of Experimental Biology 203:* 3639–3654.

Pidancier, N., C. Miquel, and C. Miaud. 2003. Buccal swabs as a non-destructive tissue sampling method for DNA analysis in amphibians. *Herpetological Journal 13:* 175–178.

Sexton, O. J. 1962. Apparent territorialism in *Leptodactylus insularium* Barbour. *Herpetologica 18:* 212–214.

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

Wright, K. N. and B. R. Whitaker. 2001. *Amphibian Medicine and Captive Husbandry*. Malabar, Krieger Publishing Company. 570 pp.