

SHORT COMMUNICATION

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

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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 Whitaker 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 Whitaker 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 (Departamento 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

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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



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

(*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.

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