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# An Improved Blood Sampling Technique for Hatchling Emydid Turtles

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Blood sampling is useful for the study of behavior, ecology, and physiology. It is a minimally invasive method of obtaining tissue that allows, for example, genetic, toxicological, and stable isotope analyses. Various techniques for obtaining blood from turtles have been described (Avery and Vitt 1984; Bennett 1986; Owens and Ruiz 1980; Rogers and Booth 2004; Wibbels et al. 1998; reviewed by Kutchling 1999). Most published techniques apply to adult turtles, with the exception of Bennett (1986) and Wibbels et al. (1998), who described blood sampling from the dorsal cervical sinus and the subcarapacial vein of hatchling sea turtles. We are unaware of a blood-sampling technique for hatchling emydid turtles, which are smaller (usually < 10 g) than cheloniid hatchlings (≈ 20 g). Although we found the technique reported by Wibbels et al. (1998) to be useful on smaller emydid hatchlings, we successfully used an alternative technique that we believe is more appropriate and less detrimental. Here we describe this technique, report our success, and discuss its advantages over the previously described techniques.

In summer 2005, we collected 34 gravid female Common Map Turtles (*Graptemys geographica*) at a nesting site in Lake Opinicon (150 km S of Ottawa, Ontario) and induced oviposition with oxytocin (10 IU/kg) (Ewert and Legler 1978). We incubated eggs in the laboratory at 29°C and we obtained blood on all hatchlings (N = 338) to determine paternity. Hatchlings weighed 4.7–10.3 g (mean = 7.5, S.D. = 1.1 g). We collected between 0.03 and 0.05 ml of blood with a 0.5 ml insulin syringe (B-D U-100) fitted with a 13mm long (28.5 ga) needle. We obtained blood from the coccygeal vein, which runs along the dorsal midline of the tail (Fig. 1). The venipuncture site was between the cloaca and the base of the tail, along the dorsal midline (common map turtles have a yellow stripe running along that midline). The needle was directed cranioventrally at a 45° angle and inserted approximately 2 mm deep between two adjacent vertebrae. If the needle went deeper than 2 mm, it meant that the needle was beside the vertebrae and no blood could be obtained. As soon as the opening of the needle was in the skin, the plunger of the syringe was withdrawn gently (up to the 0.2 ml mark) to create a vacuum. Care was taken not to apply excessive negative pressure to prevent collapse of the vein (Rogers and Booth 2004). If blood did not appear in the syringe immediately, we slowly rotated the syringe and/or gently increased or decreased the angle until blood was withdrawn into the syringe. If an insufficient amount of blood was obtained on the first try, we made a second attempt a few mm closer to the base of the tail. If no blood was obtained on the second attempt, however, we sampled the subcarapacial vein (a branch of the external jugular according to Rogers and Booth (2004)) as described by Wibbels et al. (1998) to avoid damaging the tail blood vessels. For 248 of the 338 samples taken, we recorded the presence of extracellular fluid, the number of attempts (up to two), and the volume of blood taken in each attempt.

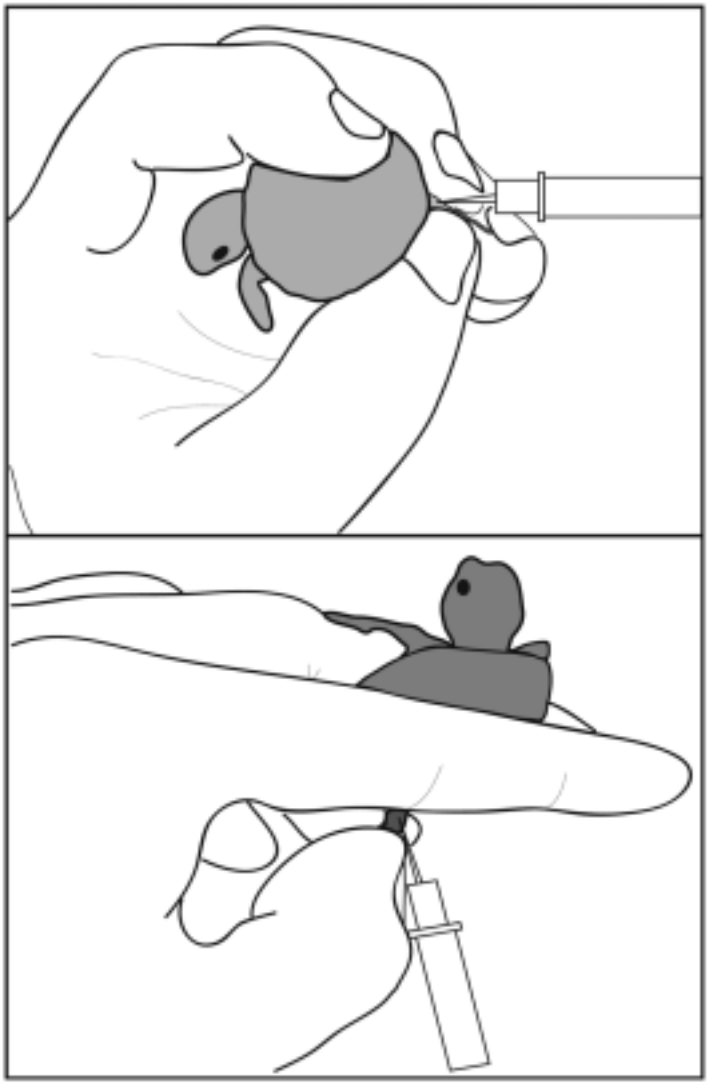

FIG. 1. Two ways of handling hatchling turtles for sampling blood from the coccygeal vein.

In a maximum of two attempts, we were successful in taking at least 0.03 ml (mean = 0.047 ml) of blood from the coccygeal vein for 83% of the hatchlings. For the remaining 17% of the hatchlings, we obtained the blood from the subcarapacial vein (also in a maximum of two attempts). Our success at obtaining blood from the coccygeal vein was independent of hatchling size (logistic regression with carapace length as the independent variable: $\chi^2 = 0.0012$, $P = 0.97$). This result suggests that the technique is practicable on hatchlings of smaller species, such as painted turtles (*Chrysemys picta*) or spotted turtles (*Clemmys guttata*) that overlap in size with the smaller hatchlings of common map turtle (Ernst 1994). However, this technique may difficult to apply on species with relatively short tail.

We noted the presence of extracellular fluid or lymph in 16% of the samples from the coccygeal vein, but in 75% of those samples

it was estimated to be ≤ 0.01 ml. In the samples from the subcarapacial vein, extracellular fluid or lymph was noted in 58% of the samples and exceeded 0.01 ml in 86% of those samples. Extracellular fluid and lymph are undesirable in blood samples for both genetic analysis and haematological studies (Lopez-Olvera et al. 2003). We released most turtles 1–3 weeks after blood sampling and none died or showed signs of weakness while in captivity. Another 120 hatchlings were kept in the laboratory for more than a month and none died.

We believe that the coccygeal vein should be preferred over the subcarapacial vein for venipuncture in hatchlings for three reasons. First, bleeding never occurred when we sampled from the coccygeal vein, whereas occasional bleeding occurred when we took blood from the subcarapacial vein. The subcarapacial vein is more likely to produce abundant bleeding because it is a larger vessel. Second, we obtained extracellular fluid more often and in greater amounts when we used the subcarapacial vein. Finally, vital organs are less likely to be injured when blood is taken from the dorsal side of the tail rather than from the head and neck regions.

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# Retention Rates of Surface and Implantable Marking Methods in the Mediterranean House Gecko (*Hemidactylus turcicus*), with Notes on Capture Methods and Rates of Skin Shedding

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Many ecological studies of amphibians and reptiles commonly use toe clipping to permanently mark small species. However, researchers have noted deleterious effects on locomotion and even survival caused by toe clipping (Bloch and Irschick 2004; Clarke 1972; Dodd 1993). Alternatives to toe clipping include implantable and surface-marking techniques, yet the former requires the species of interest to have transparent or semi-transparent skin, and the latter may be highly susceptible to wear or loss by shedding.

The Mediterranean House Gecko (*Hemidactylus turcicus*) is an introduced species that is found in association with human habitation in many regions of the United States (Selcer 1986), including Tucson, Arizona where we conducted our study. In our experience, simple surface-marking techniques (permanent marker, correction fluid, etc.) are inadequate for monitoring individual *H. turcicus* because these lizards shed frequently. Our goal was to provide a permanent marking technique for individual *H. turcicus* for periods in excess of 2 wk to facilitate population monitoring.

Based on our observations, the variation in dorsal patterns is not readily distinguishable among individual *H. turcicus*. For small species like *H. turcicus* (SVL = 44–59 mm; body mass = 2.1 g), external tags are often not appropriate. However, internal microtagging has been used successfully in a number of studies (e.g., Buckley et al. 1994 for fish). Here, we use implantable elastomer tags (Northwest Marine Technology: LOCATION NEEDED) to mark small geckos. This method uses a two-part silicone-based material mixed immediately before use, which is injected subcutaneously. Injected as a liquid, it soon cures into a pliable, biocompatible solid that remains visible under translucent skin. In *H. turcicus* the best tagging area was the ventral skin surrounding the leg joints because the dorsal surface has a darker, leopard-like pattern. Tag visibility can be enhanced by fluorescing the marker with a blue-light LED or UV light. A single color kit, including the injector, a 5-cc tube of elastomer, mixing supplies, blue LED flashlight with amber glasses, carrying case, and instructions costs U.S. $215, although more complicated kits cost significantly more. These implantable microtags have been used in ecological studies of crustaceans (e.g., Godin et al. 1995), fish (e.g., Goldsmith et al. 2003), amphibians (e.g., Pfennig and Murphy 2000), and reptiles (e.g., Losos et al. 2004).