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A PIT Tagging Technique for Ambystomatid Salamanders

The ability to accurately and efficiently create individual marks on sample organisms is critical for biologists interested in studying a variety of ecological, evolutionary, behavioral, and conservation questions in a range of animal species (e.g., Murray and Fuller 2000; Hagler and Jackson 2001; Gibbons and Andrews 2004). Among herpetofauna, PIT (passive integrated transponder) tag technology has been successfully utilized as an individual marking technology for years in a number of species (Baker and Gent 1998; Gibbons and Andrews 2004; Ferner 2007). Techniques for implanting PIT tags vary, particularly in amphibians (Ferner 2010). Such techniques can be limited in terms of their efficiency in time and money, and often differ in terms of probability of infection, mortality rates, and tag loss (Ott and Scott 1999; Ireland et al. 2003; Ferner 2010).

As part of our use of PIT tags in a long-term mark and recapture study of Arizona Tiger Salamanders (*Ambystoma tigrinum nebulosum*; Whiteman and Wissinger 2005; Wissinger et al. 2010; Whiteman et al. 2012), we developed a PIT tagging technique that is quick, requires no anesthesia, produced no observed mortality when correctly performed, potentially provides 100% tag retention, and can be performed in the field. A short video of the technique is available at: http://campus.murraystate.edu/academic/faculty/hwhiteman/video.shtml.

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Our method begins with successfully positioning a salamander in the hand such that the head, tail, legs, and body are controlled and not allowed to move, with the left side of the salamander's body exposed. It may be easier for some researchers to use the right side; additionally, researchers with smaller hands tend to manipulate the salamanders in different ways than shown in the video. The major goals, however, are to expose the lateral midsection of the animal while keeping the animal from moving during the PIT tagging process.

We then count five to six costal grooves anterior to the rear legs, and place a 1.5-mm incision between and parallel to the costal grooves approximately midway between the dorsum and abdomen using a sterilized (70% ethanol) scalpel (see video and Fig. 1A). This incision only cuts through the epidermis and does not enter the muscle layers. Next, we insert a sterilized flat needle housed on a VIA (Visible Implant Alpha; Northwest Marine Technology, Inc., Shaw Island, Washington) implanter into the incision and push the needle posteriorly between the epidermis and muscle layers, parallel to the body (Fig. 1B). The flat needle improves upon the larger round needle used in standard PIT tag implanters by minimizing the furrow created between the epidermis and the abdominal wall. We insert the needle to a point approximately medial between the next two costal grooves, slightly bend the needle, and push so that it gently pierces the body cavity underneath the epidermis. In this way we create a furrow for the entry of the PIT tag, and assure that the opening to the body cavity is not the same as the one in the epidermis, potentially minimizing infection, hernia, and tag loss.

After removing the flat needle, we insert the sterilized PIT tag (12.5 mm; Biomark, Inc., Boise, Idaho) into the incision using our fingers and slide it along the furrow to ensure it follows the correct path (Fig. 1C). As the PIT tag approaches the opening to the body cavity, we tilt it slightly and push it downward into the body cavity. On occasion the PIT tag must be manipulated multiple times to find the entry hole into the body cavity, and, more rarely, a second hole is created near the first one using the

PHOTOS BY SARAH THOMASON

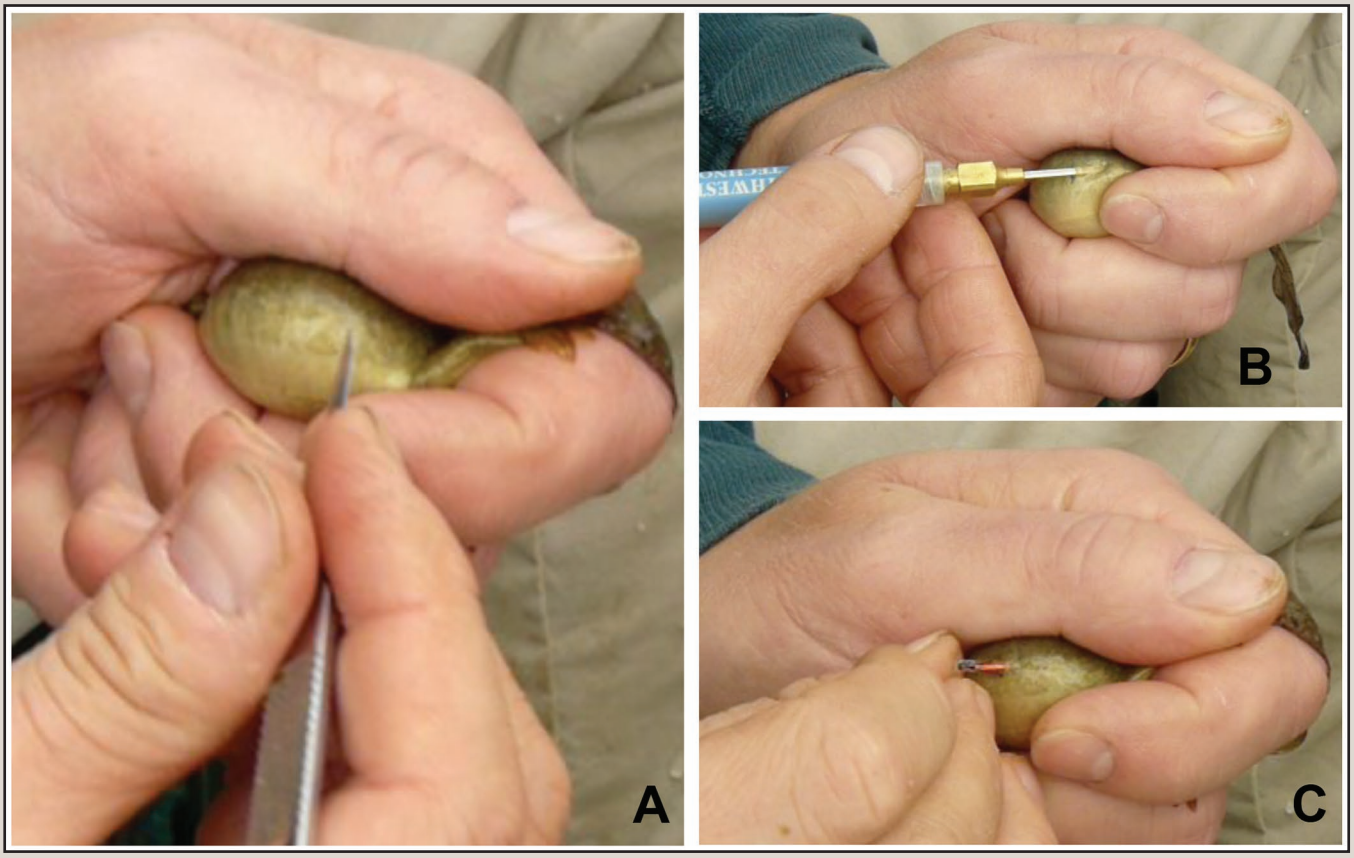


FIG. 1. PIT tagging technique developed for ambystomatid salamanders. A) An epidermal incision is created between and parallel to the costal grooves approximately midway between the dorsum and abdomen using a sterilized scalpel. B) A sterilized flat needle housed on a VIA implanter is inserted into the incision and pushed posteriorly and parallel to the body between the epidermis and muscle layers. The needle is inserted to a point approximately medial between the next two costal grooves, slightly bent, and pushed to gently pierce the body cavity beneath the epidermis. C) After removing the needle, a sterilized PIT tag is inserted into the epidermal incision, slid along the furrow, and then tilted and pushed into the body cavity. See text for further details of the procedure.

flat VIA needle. We then use a cotton swab dipped in Bactine to sterilize the incision, and release the animal into a holding tank. Animals are briefly observed and then released en masse with other PIT-tagged individuals after all animals have been processed.

The entire procedure takes less than two minutes, and with practice considerably less time. Although salamanders feel momentary pain during the surgery, their behavioral responses suggest that it is less painful than what they sometimes experience during toe clipping. Anesthesia is thus not needed, unlike some previous PIT tagging techniques (e.g., Ott and Scott 1999; Ryan et al. 2014), and in fact would make the implantation more difficult, as the positive pressure exhibited by the salamander when held is helpful for successfully implanting the PIT tag.

Tag retention was 100% in overnight (N = 10) and 10-day laboratory studies (N = 20) using this technique. Although we have not systematically evaluated tag retention over longer periods of time, our two-incision procedure without the use of a PIT implanter makes it highly unlikely for PIT tags to back out of the second incision, as tags are pushed completely into the body cavity, and positive pressure from the epidermis typically keeps the incision sealed. This technique thus improves upon single-incision techniques that have exhibited 25–45% PIT tag loss in congeners (Hamed et al. 2008; Ryan et al. 2014).

We have utilized this procedure as our primary marking method for our study population between 2005–2015. We have not observed any mortality during overnight observations of implanted salamanders, 10-day laboratory trials, or as evidenced by the lack of observations of dead animals in ponds days to weeks after PIT tagging. Because our study ponds are oligotrophic and clear, dead salamanders are obvious (Wissinger and Whiteman 1992; Whiteman and Wissinger 2005). Hundreds of PIT-tagged animals have now been recaptured, with many recaptured multiple times over the ensuing years. Additionally, we have observed no infections around incisions; recapture of individuals has revealed that incisions heal within a few weeks, and incision scars are often not visible the following year. A rigorous test of the survivorship of PIT-tagged versus toe-clipped animals is currently ongoing.

In summary, this technique allows permanent placement of PIT tags within the body cavity while minimizing the potential for infection or increased mortality. We have successfully used this technique on larval (as small as 56 mm SVL), metamorphic, and paedomorphic tiger salamanders, and we predict it will work similarly in other ambystomatids, as well as other appropriately sized salamanders and anurans with proper modification.

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