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Comparison of Immobilization Methods for PIT Tagging Three-toed Amphiuma (*Amphiuma tridactylum*)

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Amphiuma tridactylum is a large (up to 1 m and 1 kg), slimy, aquatic salamander that can deliver a powerful bite, and thus is difficult and potentially dangerous to handle (Fontenot Jr. 1999; Fontenot Jr. and Seigel 2008). Even a simple task like PIT (Passive Integrated Transponder) tag implantation is virtually impossible without restraining the animal. We have observed that forced physical restraint is undesirable because it can result in skin abrasion (from gloves or a towel), physical trauma to internal organs (e.g., liver damage), and potentially death. Recently, Brown and Forstner (2009) reported a method of handling *Amphiuma* in the field using snake restraining tubes. We found this method difficult to implement with *Amphiuma*, especially when carefully examining specimens for bites. Burgmeier et al. (2010) developed the "bender" board for restraining Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis)*. We sought a quick method of temporarily immobilizing *A. tridactylum* individuals for examination and PIT tag implantation, comparing one method of anesthesia (isoflurane) and immersion in a cold-water bath. Alamndarz (1975) used cold water to aid transferring adult crocodilians, and Mitchell (2009) refers to cooling as a method of minimizing movement with amphibians. Mitchell (2009) discusses several anesthetics for amphibians. We selected isoflurane because preliminary trials with *A. tridactylum* individuals indicated it was safe and effective.

Methods.—Eighteen adult *A. tridactylum* were collected with baited traps (flexible fish traps; Promar 36" x 12" with ¼" mesh) baited with raw beef heart, chicken livers or most effectively, canned cat food) from a ditch and small pond in East Baton Rouge Parish, Louisiana, USA. The specimens were maintained for 1–3 days in the laboratory in aquaria at 25°C and fed one earthworm per day each.

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We used isoflurane anesthesia for three of the specimens. Isoflurane anesthesia methods were those of Major et al. (2011)

FIG. 1. A Three-toed Amphiuma being weighed after cooling in ice water.

FIG. 2. A Three-toed Amphiuma after cooling in ice water. It is possible to examine the specimen leisurely, even to the point of placing them ventral side up.

and are only summarized here. We placed each individual into a body-size matched polycarbonate snake tube. We placed a cotton ball saturated with 0.2–0.5 ml (as needed) of isoflurane into head the end of the tube and plugged the ends with rubber stoppers.

Fifteen specimens were immersed in a container of water and ice for ten minutes or until they did not exhibit a righting response.

Prior to insertion, PIT tags and the syringe needle were dipped in 90% isopropyl alcohol to ensure aseptic conditions. While under anesthesia, with the individual in the clear plastic tube, the posterior third of its body was pulled from the tube, and the tail was held with a dry cloth. The PIT tag was inserted subcutaneously on the right side (dorsal view) of the body approximately 5 cm anterior to its cloaca, with the syringe provided by the manufacturer (Avid Identifications Systems, Norco, California). We inserted the syringe subcutaneously slightly farther than necessary, so as to leave ~ 1 cm of space between the PIT tag and the puncture made by the syringe, to allow subcutaneous space for fascia reformation. The insertion hole was then sealed with New Skin (solution of -Pyroxylin, Alcohol, Oil of Cloves, and 8-hydroxyquinoline).

Animals anesthetized with isoflurane were limp during and after PIT tag implantation, and the skin surface showed visibly dilated blood capillaries. When individuals were placed in water for recovery, we were concerned that they may drown, and so supported their heads above the water surface until they began moving on their own.

Because respiratory rate is reduced during anesthesia, and respiration is the primary method of voiding the isoflurane anesthetic, recovery can be facilitated by manually forcing expiration (inspiration then follows naturally by elastic recoil). Forced expiration can be induced with the investigator's thumb by pressing on the ventral side of the subject, beginning just anterior to the cloaca, and drawing the thumb in a posterior to anterior direction as far as the pectoral girdle. The lungs are basically hollow sacs that extend most of the length of the peritoneal cavity, so the sequential compression motion described above essentially squeezes the air out. This recovery facilitating method is effective, but applying it may be limited by the nature and location of the incision, etc.

Results.—For specimens subjected to isoflurane, anesthesia was achieved with less than 20 minutes of exposure, as indicated by lack of righting response and muscular tension. Recovery to the point of voluntary movement and regular breathing generally took 30–60 min, depending on the individual. However, one individual showed little or no reviving at 60 min, but was allowed to remain in shallow water, and eventually recovered after 4–6 h. Specimens subject to cold treatment retained a firm body and muscular tension. The skin surface showed visibly dilated blood capillaries, even more so than with the isoflurane treated individuals. However, the cold-treated individuals began moving on their own within one minute when placed in ambient temperature water, and showed no negative symptoms. They were able to swim and breathe as normal in 3–5 minutes, and cutaneous blood capillaries returned to normal condition within 30 minutes.

Discussion.—Both methods were effective for immobilizing *A. tridactylum*. However, the utility of each is dependent on the type of procedure being done, the amount of time required for the procedure, and the amount of time available for application of the anesthesia/immobilization procedure, recovery time, and post-procedure monitoring.

General anesthesia with isoflurane is suitable for procedures requiring a working time of 20+ minutes. This method can also work satisfactorily for short duration procedures, but it requires an extensive amount of time for application, monitoring, and recovery. Hence, its use may be impractical, particularly if a quicker temporary immobilization method is available.

The process and recovery time required for cold immobilization/narcosis may be most practical for use with a quick procedure like PIT tag implantation, which generally takes less than one minute. Applying cold to immobilize was quick and easy, as was recovery. Although it is not a replacement for chemical anesthesia when a long working procedure time is required as with general surgery, e.g., with radio transmitter implantation, we believe this method will useful to other researchers who need to perform simple procedures on difficult to handle amphibians such as *A. tridactylum*.

Acknowledgments.—The methods used in this work follow guidelines of the Society for the Study of Amphibians and Reptiles (http://www.asih.org/files/hacc-final.pdf) and were approved by the Animal Care Committee at SLU (IACUC, Protocol #0015) and LSU (protocol number 09-072).

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