An Improved Technique for Formalin Fixation of Large Vertebrates

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While injection of formalin solution by hand-held syringe (Anderson 1965; Simmons 1987) is easy and effective, it can be both inefficient and laborious when preserving larger specimens. Turtles are especially difficult to prepare and they are often inadequately preserved because of the difficulty with injecting sufficient preservative into the body cavity. Turtles preserved using hand-held syringes seldom have everted penes and the head and limbs often remain tightly enclosed within the shell. We improved this method by using a modified garden sprayer for the pressurized injection of formalin (Fig. 1). Using our technique, the preservation of 173 previously frozen turtle specimens required only 4 h, an average of 3 min per turtle. We estimate the time required by manual injection would have approached 22 h. Our technique resulted in the successful eversion of nearly 80% of the penes and produced effective limb and neck extension in 95% of specimens.

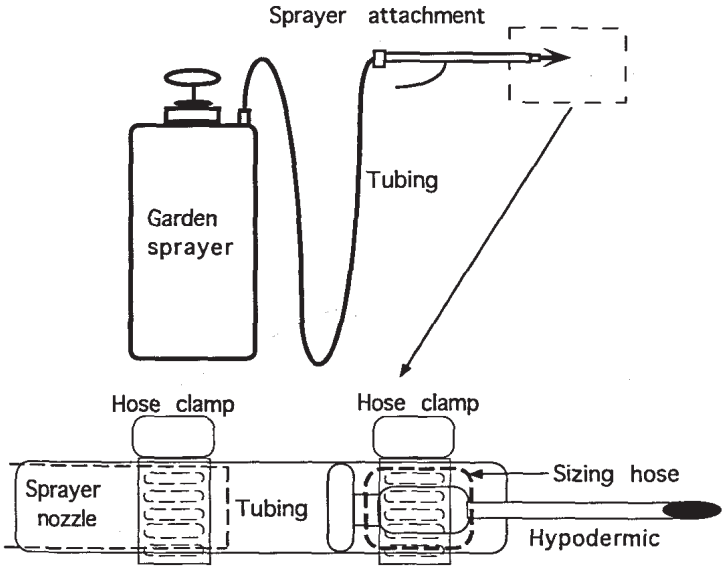

FIG. 1. Pressurized formalin injection sprayer. Dashed box provides alignment and assembly component detail for the hypodermic attachment.

The sprayer (Fig. 1) is available at most hardware stores; currently we use a Root:Lowell Heavy Duty Model (No. 1996) with a brass nozzle. Other materials required include tubing (Fisher 14-176 series), injection needles (stainless steel Fisher 14-825 series), and an assortment of hose clamps (Fisher 14-198 series). The needle assembly consists of clear, chemical-resistant, tubing (12–16") with an appropriate gauge needle inserted and clamped at one end. This assembly may require additional smaller-diameter tubing for accurately "sizing" smaller gauge needles and assuring a pressure-tight seal (Fig. 1). Assembly is straightforward: clean the sprayer tank with a hot water rinse, remove the nozzle from the sprayer delivery wand, and attach the hose assembly.

The entire apparatus can be modified from one gauge needle to the next in a few minutes. We have found that an assortment of hose and needle combinations, chosen to fit a variety of vertebrates, is most useful. The entire cost of the unit is less than US $50. All museum and field assistants must wear proper eye and clothing protection when using the formalin sprayer.

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Surgical Procedure for Radio Transmitter Implantation into Aquatic, Larval Salamanders

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Radiotelemetry has become an important technique for studying species movements and, while externally-attached transmitters have been used on amphibians (Richards et al. 1994), our initial experiments with harnesses on *Dicamptodon tenebrosus* resulted in harness slippage and skin irritations. Transmitters have been implanted effectively in several amphibian species (Richards et al. 1994) and implantation methods for large aquatic salamanders have been described (*Cryptobranchus alleganiensis*; Stouffer et al. 1983). Here we describe the surgical method (modified from Stouffer et al. 1983) we used for larval *D. tenebrosus*, translocated to the upper Sacramento River from connecting tributaries in Siskiyou and Shasta counties, California, USA, and discuss factors affecting the incision healing rate of an aquatic salamander.

During May and June, 1995, 19 *D. tenebrosus* larvae (156–233 mm total length, 39.5–72.0 g) were captured; transmitter weight (2.5 g) was limited to < 6.5% of body weight. All 19 salamanders were implanted with SM1 transmitters (2.5 g, 8 mm x 10 mm 21 mm, AVM Instrument Co.) that had been potted in dental acrylic. Salamanders were anesthetized by submersion in a 0.01% solution of benzocaine. Anesthetized animals were then placed right side down in a foam-lined plastic tray saturated with 0.01% benzocaine solution. The foam lining kept the salamander's skin moist and prevented the animal from sliding during surgery. To keep the

skin moist and ensure continued anesthetization, several pieces of anesthetic-soaked cotton gauze were placed over the animal's body so that only the flank was exposed.

An 8–10 mm incision was made mid-laterally in the left flank to reduce the incision site's contact with substrate or moving limbs during normal locomotion while healing. To initiate the incision, a pinch of skin was raised with forceps from underlying muscle and cut cranio-caudally with sharp-tipped scissors. A small hole was then punctured through the muscle and peritoneal membrane using the tips of the closed scissors and widened by opening the scissors slightly. Lifting the muscle with forceps, we extended the incision with scissors, being careful to avoid any internal organs. Sterile cotton applicators were used to absorb fluid from the body cavity or to apply pressure to bleeding vessels.

Each transmitter was dipped in molten paraffin 3 times prior to implantation to create an inert surface and to reduce the possibility of an inflammatory response. Then each transmitter was soaked for 24 h in a 5% Chlorhexidine solution (Novisan, Fort Dodge) to ensure sterility, rinsed in sterile saline solution to remove the disinfectant, and placed within the coelomic cavity (parallel to the spine) with battery oriented anteriorly and helicoil antenna dorsally.

The muscle was closed by continuous suture of 4-O or 5-O polyglactin 910 (Vicryl, Ethicon) with a taper needle; suture material was soaked in 12.9% Benzalkonium chloride (Benz-all, Xttrium Laboratories) for ca. 24 h before surgery (to soften the thread) and rinsed in saline at time of use. The skin was closed using approximately 3 horizontal mattress sutures (ca. 2 mm wide and 2 mm apart) of 4-0 or 5-0 polyglactin 910 with a cutting needle. Skin sutures were not drawn as tightly as those in muscle tissue to prevent necrosis. The closed incision site was wiped clean with gauze. Mean surgery time per individual was 21 minutes.

While nonabsorbable 4-O silk suture material has been recommended because of its cost and handling characteristics (Boothe 1985), our earlier experiments with silk suture material resulted in severe fungal infection in *D. tenebrosus* unable to shed the sutures. Silk suture material causes greater tissue reaction (Boothe 1985) and a higher frequency of infection (Sugarman and Musher 1981) than nylon suture material. We used absorbable Polyglactin 910 sutures and found them to be compatible with larval salamanders.

To resuscitate animals, recovering individuals were placed in a 5-gallon bucket of cold spring water and the water was stirred and changed frequently to increase the exchange of oxygen and dilution of the anesthetic. Recovery time averaged 27 minutes and the total procedure (anesthesia, surgery, recovery) time per individual averaged 62 minutes.

Salamanders were then held in tubs for 10–20 days to monitor their behavior and to assure that the sutures remained intact; failing sutures were replaced immediately as described above. At the time of release, we remeasured weight and snout-vent length, documented incision status, and applied malachite green, an anti-fungal agent, to the incision site.

During June and July, 1995, nine of the salamanders were translocated to the upper Sacramento River and ten were released at their points of capture. Fourteen salamanders were recaptured between September and mid-October, 1995. Incisions in 8 of the 14 recaptured salamanders were completely healed (4 each from tributaries and river), 4 were partially healed (3 from tributaries and one from river), and two were not healed (both from tributaries). Incision healing time may be affected by water temperature. Higher metabolic rates and faster healing times are associated with higher temperatures in fish (Anderson and Roberts 1975) and snakes (Smith et al. 1988); larval *Dicamptodon* body temperatures are the same as the water temperature (Brattstrom 1963) and their metabolic and healing rates are assumed to increase with increasing water temperatures.

Our observations of individuals radiotracked for a few weeks during 1994–1995 indicate that these salamanders remain fairly sedentary in winter when water temperatures are colder. One individual, implanted with a transmitter in October, 1994, and maintained in cold spring water in the lab, took six months to heal. While healing time may have been influenced by the suture material (silk), it may also have been increased by cold water temperature and the animal's inactivity during winter. Most incisions healed within three months during summer. Individuals implanted with transmitters in the summer were more active, and the water temperature was also generally warmer; we recommend timing implant surgery for aquatic salamanders to correspond with the organism's most active season. Maintaining or releasing animals in waters at the higher end of the salamander's preferred temperature range may accelerate healing.

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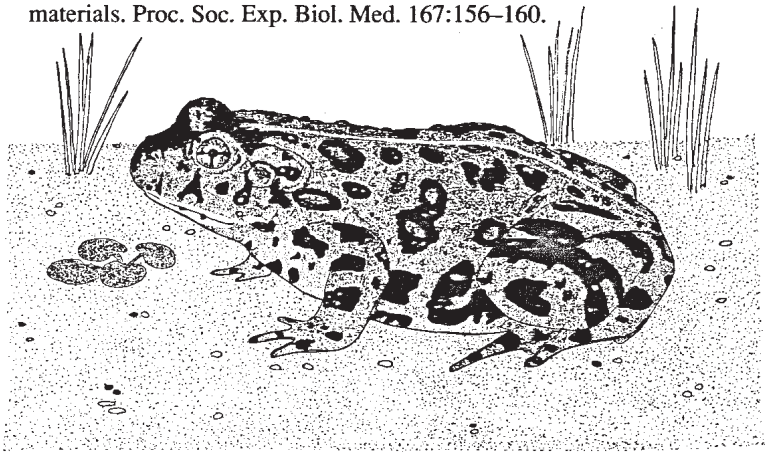

Bufo boreas halophilus (California Toad). USA: California: San Diego Co., Santa Margarita River. Illustration by Dan Holland.