Appendix 2

Techniques for Marking Amphibians

Maureen A. Donnelly, Craig Guyer, J. Eric Juterbock, and Ross A. Alford

Marking adult amphibians

Several methods are available for marking adult salamanders and anurans (see Ferner 1979). The type of system used depends on the size and natural history of the target organism as well as time and resources. Some marking methods require special equipment, training, or permits from state or national wildlife agencies. Marks can be permanent, temporary, date-specific, or individual-specific. Long-term studies of populations require that marks be permanent. Temporary marks can be used if information on behavior over the course of a day or night is required (Jacobson 1985). Date-specific marks (all animals are given identical marks during a sample period) can be applied more rapidly in the field than individual-specific marks, and it is simpler to take data from recaptured animals marked by date. Most population estimators accommodate the use of date-specific marks. If a project requires detailed information on space-use patterns or growth rates in addition to an estimate of population size, individual-specific marks must be used.

Pattern Mapping

Color patterns of some amphibians vary among individuals and are analogous to fingerprints. These patterns can be recorded or "mapped" with photographs or sketches (Forester 1977; Tilley 1977; Andreone 1986). Photographs should be sharp and shadow-free, with good color rendition. Best results are obtained when animals are anesthetized (see Appendix 1) and then photographed underwater. Several animals can be photographed together (3 to 4 adults or 8 to 12 juveniles per photograph), each being assigned a two-part number (#A-#B) to denote photograph (A) and position (B). Photoidentification requires a camera body, a macro lens, lighting (flash/strobe), film, and film processing. Color film provides the most realistic record of animal pattern, but if an animal's colors are contrasting, black and white film is a less expensive alternative. In field situations, Polaroid cameras allow for immediate results, but resolution is poor. Sketching animals requires more time but is less expensive than photography.

Loafman (1991) described a technique analogous to pattern mapping for spotted salamanders, which may be suitable for any spotted amphibian. He described each animal's pattern as the number of spots found on defined parts of the body (for example, head, neck, body, limbs). Loafman grouped all animals with the same head-spot pattern together. Within each head-spot group, animals were further classified by snout-vent spot counts, neck spot counts, and sex.

Marking and Tagging

Both tags and marks are permanent. They vary, however, in ease of application, cost, and potential negative effects on study organisms. Ideally, animals should be marked in the field and released at their capture point. If animals must be transported to a laboratory or field camp for marking, they should be returned to their capture point as soon as possible. Most tagging methods presently available probably work best for medium-sized to large frogs. We discuss several methods here. Radioactive and radiotelemetric marking are described in Chapter 7.

TAGS

Raney (1940), adapting a jaw tag used for fish (Shetter 1936), attached Monel metal strap or clip tags to the lower jaw of frogs with pliers. He recommended number 3 tags for adults and number 2 (fingerling) tags for juveniles. Raney (1940) asserted that the tags did not cause infection, but he did note that frogs pulled at the tags with their forelimbs, although the flesh did not tear. Stille (1950) reported tag loss, and Stebbins (*in* Woodbury 1956) observed irritation at the site of tag attachment. Kaplan (1958) tagged frogs with small, numbered, aluminum bird bands (butt-end) placed around the outer toe. The cylinder was closed with pliers only tightly enough to secure the band, but the toe webbing was pierced. Both Monel metal strap and butt-end bird bands are available from National Band and Tag Company (Appendix 6).

Breder et al. (1927) attached tags to cords that were tied around the waist of the frog just in front of the hind limbs. The string was tied tightly enough to prevent tag loss; when it was too tight, it wore through the skin (Breder et al. 1927). Cardboard tags, which lasted approximately three months in the field, were attached with soft monofilament line. Aluminum tags were stamped with letter punches and attached with enameled trout line. For small species, tags consisting of three different-color beads were attached with a silk thread. Savage (1934) attached a small, waterproof, numbered paper tag to the waist of each frog in a field study of breeding behavior. He observed some tag loss and associated it, in part, with a decrease in female size after oviposition. The tags did not adversely affect breeding behavior.

Waistbands made from preshrunk nylon elastic banding that was painted and numbered were used to tag amphibians for behavioral studies (Woodbury 1956; Emlen 1968). The colored bands were visible from a distance of more than 5 m. The bands were tight enough not to catch on vegetation but loose enough to permit normal activity. Bands of this type are probably best used in short-term studies and must be removed at the end of the study.

Elmberg (1989) followed 637 *Rana temporaria* that had Floy-T tags (FT F-69 Fingerling tag) tied to their knees with elastic thread. These tags (6 × 3 mm), designed originally for small fish, come with thread and needle (tags available from Floy-Tag and Manufacturing; see Appendix 6). Tag loss ranged from 3% to 15% and resulted largely from imperfect application (Elmberg 1989). This method can be used for any frog or toad whose knee is narrower than the upper and lower legs. The size of the tag relative to the size of the frog must be considered, and this method may not be useful for small species or for juveniles of large species.

Nace and Manders (1982) used glass beads to identify individual *Xenopus* in laboratory populations. They anesthetized the animals (see Appendix 1) and inserted a 21-gauge hypodermic needle through the upper arm (lateral to the humerus) or leg (medial to the femur). A surgical wire (Ethicon Sutupak 0000 [32-gauge] or 00 [28-gauge] surgical steel monofilament Type B noncapillary) was passed through the needle, and the needle was withdrawn. A sequence of beads was strung on the wire, which was secured by tying a square knot on the medial side of the upper arm or the lateral side of the leg. The humerus or femur anchored the bracelet. This type of marking device should be tested before it is used in the field to ensure that the bracelet does not snag surrounding vegetation. In the laboratory, some frogs maintained their marks for more than 3 years.

POLYMERS AND PIGMENTS

Woolley (1973) tagged salamanders (*Eurycea lucifuga* and *E. longicauda*) with acrylic polymers. He filled a 2-cc syringe fitted with a 22-gauge hypodermic needle with a mixture of two parts acrylic polymer (Liquitex) and one part distilled water. He injected the mixture into the tail of each salamander until a mark 7 to 10 mm wide was obtained. The marks were distinct for 19 months and visible from a distance of 3 to 5 m.

Fluorescent pigments have been used to mark fishes (Phiney et al. 1967; Phiney and Mathews 1969), and some researchers have adapted these techniques to mark amphibians. Taylor and Deegan (1982) sprayed fluorescent pigments into the skin of aquatic amphibians (adults and larvae) with pressurized air (using a spray gun) to mass-mark individuals. Nishikawa and Service (1988) modified this technique to mark individuals of terrestrial species. They reduced the inner diameter of the spray gun nozzle to increase mark density, reduce mark size, and improve control over mark location. With the Nishikawa and Service technique, marks can be applied in the field without anesthesia, recapture rate is high, and marks are visible in light or dark. A portable ultraviolet light source is required for nighttime identification of individuals marked with fluorescent pigments. All supplies used in fluorescent marking (including four inert, nontoxic pigments) can be obtained from Scientific Marking Materials (Appendix 6). Fluorescent pigments (nine colors) are also available from Radiant Color Company (Appendix 6).

For salamanders, 10 mark locations (five on each side: anterior and posterior to arm, midbody, anterior and posterior to leg) coupled with five colors can be used to mark millions of salamanders (Nishikawa and Service 1988). It may be possible to mark small individuals in only three or four locations. Marks can last up to 2 years, but the survival rates of marked animals relative to those of animals marked with other techniques are not known.

Ireland (1991) described another fluorescent marking method, less expensive than those that use pressurized air. A probe dipped in glycerol and fluorescent powder is pressed on an area of skin that has been roughened with a pencil eraser. Several animals can be marked by varying color and position. The marks last approximately 6 months.

TRANSPONDERS

A passive integrated transponder (PIT) is a radio-frequency identification tag that consists of an electromagnetic coil, tuning capacitor, and microchip encased in glass. It is small (10×2.1 mm, 0.05 g) and carries a 10-digit hexadecimal number that is read with a portable scanner.

Camper and Dixon (1988) implanted PITs intraabdominally (with a modified metal syringe and a no. 12 cannula) into frogs with snout-vent lengths (SVL) of 80 mm or greater. The PIT and implanter were cleaned with 70% ethanol before implantation; after implantation the site was cleaned with ethanol and sealed with Krazy Glue (a brand of superglue). The PIT was detected most easily when it was oriented parallel to the main body axis. To implant large numbers of tags, a spring-loaded syringe with a trigger can be used (available from Destron/IDI vendors; see Appendix 6). Sam S. Sweet (pers. comm.) inserts PITs into the dorsal lymph sacs of toads (SVL > 40 mm) to avoid potential damage to internal organs. He uses scissors to make a posteriorly directed nick in the skin anterolateral to the sacrum; the PIT is inserted into the opening. The PIT tag method is appealing because up to 34 billion unique codes are possible, but the method is expensive. PIT tag vendors are listed in Appendix 6.

TOE CLIPPING

The least expensive option for marking anurans and salamanders is to remove toes in unique combinations. Alford et al. (unpubl. data) marked more than 12,000 *Bufo marinus* and hundreds of native frogs in Australia by clipping toes. The technique requires good scissors and alcohol to clean them. Antibiotic or antifungal creams or powders can be applied to digits to reduce the probability of infection. Martin and Hong (1991) successfully used Bactine® to treat wounds in captive amphibians; this antiseptic could be applied to clipped digits to prevent infection. For several salamander species, toes regenerate quickly and should be cut at an angle so that regenerating digits grow back at an angle. Salamanders should be anesthetized (Appendix 1) prior to toe clipping; anurans do not need to be anesthetized, but a local anesthetic can be used.

A variety of coding schemes for use with toe clipping have been developed (Figs. 28 and 29). In

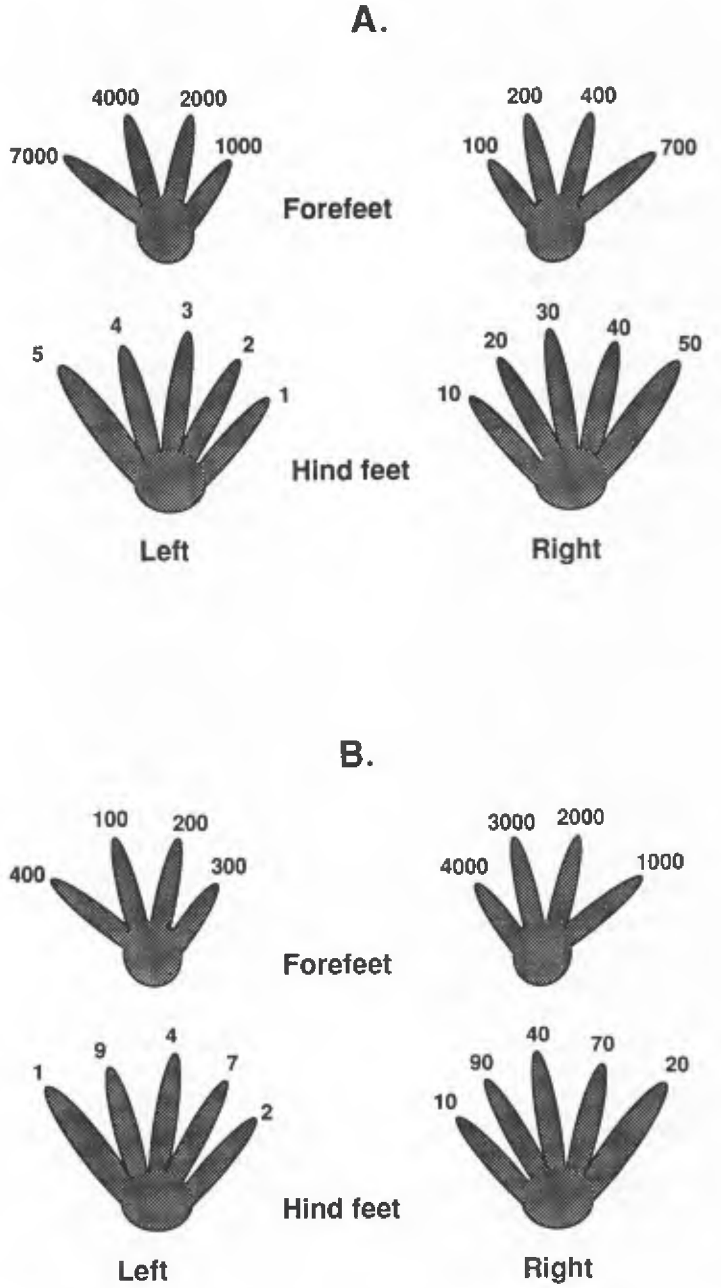

Figure 28. Clip-code schemes for marking salamanders. A. Twitty (1966) scheme. B. Scheme used by David B. Wake (pers. comm.). Using the Twitty scheme, code 4967 would require clipping one toe on the left forefoot (4000), two toes on the right forefoot (200 and 700), two toes on the right hind foot (20 and 40), and two toes on the left hind foot (5 and 2). Using the Wake scheme, code 4967 would require clipping one toe on the right forefoot (4000), three toes on the left forefoot (400, 200, and 300), two toes on the right hind foot (40 and 20), and one digit on the left hind foot (7).

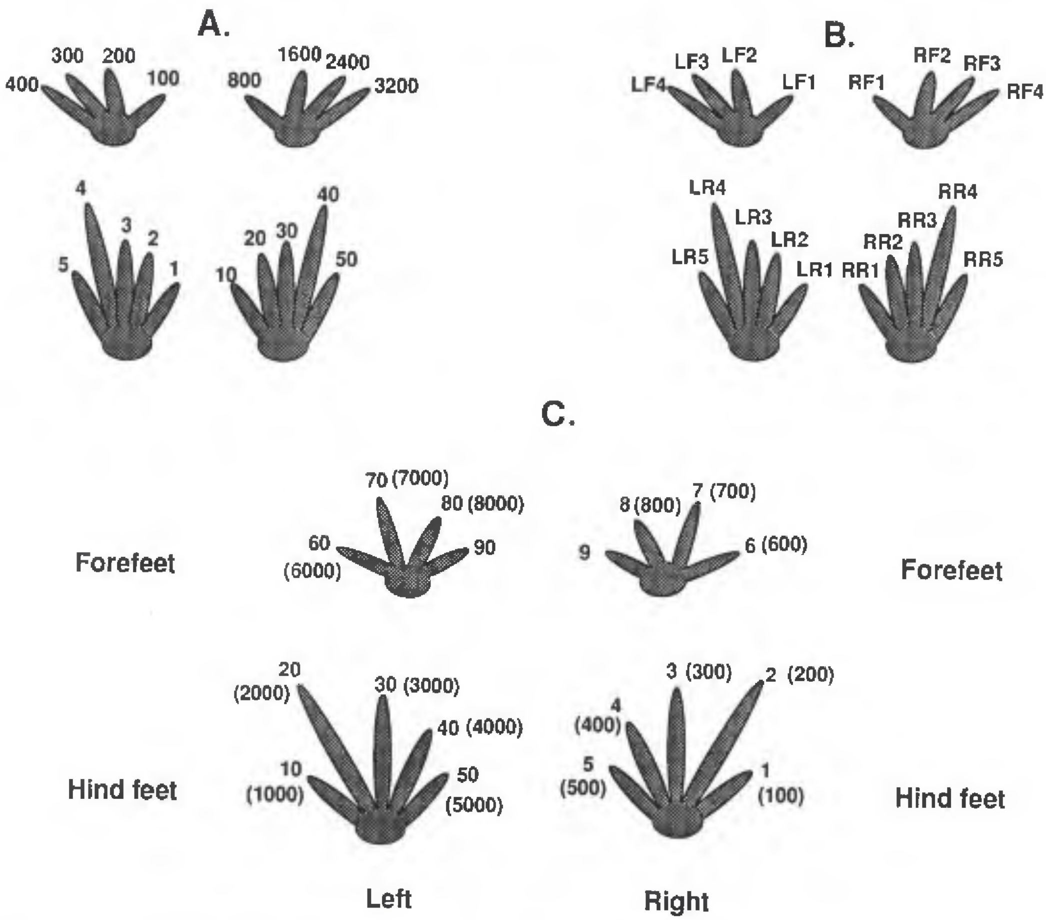

Figure 29. Clip-code schemes for marking frogs. A. Martof (1953) system. B. Donnelly (1989) system. C. Hero (1989) system. Using the Martof system, code 4967 would require clipping two toes on the right forefoot (3200 and 1600), one toe on the left forefoot (100), two toes on the right hind foot (40 and 20), and two toes on the left hind foot (2 and 5). See text for explanation of the Donnelly scheme. See text and Table 28 for explanation of the Hero scheme.

most, toes represent numbers. In the Martof (1953) scheme, for example, to mark animal number 577, two toes from the right forefoot and two toes each from the right and left hind feet must be cut (Fig. 29A). Hero (1989) devised a system (Fig. 29C) that requires clipping fewer toes than does the Martof scheme. Eighteen unique marks result when one toe is clipped, 124 marks when two toes are clipped, and 736 marks are obtained by clipping three toes (Table 28). Not all consecutive numbers are available after 99 in the Hero scheme. When two toes are cut on the same side of the body, the lower number is used; for example, if the third and fifth digits are clipped from the right hind foot, the code is 103 rather than 301 (Fig. 29C)—clip code 301 does not exist in the Hero scheme (Table 28).

In the Donnelly (1989) scheme (Fig. 29B), toes are clipped in sequence, but they do not correspond to numbers. The code associated with each toe refers to foot location (left, right, front, rear) and toe position (innermost = 1, outermost = 4 or 5). The first 18 animals have one toe excised (LF1, LF2, LF3, LF4, RF1, RF2, . . ., RR3, RR4, RR5). After all unique one-toe codes are used, two toes are excised in unique combinations. For example, the first toe on the left front foot is cut (LF1) and one sequential digit on a remaining foot is cut (LF1-RF1, LF1-RF2, LF1-RF3, . . ., LF1-RR5). After code LF1-RR5 is

Table 28. Clip-Code Numbers Available for Marking Individuals, Using the Hero (1989) Toe-Clipping System[a]

Number of toes clipped	Clip-code number
1	1–10, 20, 30, 40, 50, 60, 70, 80, 90
2	11–19, 21–29, 31–39, 41–49, 51–59, 61–69, 71–79, 81–89, 91–99, 102–109, 203–209, 304–309, 405–409, 506–509, 607–609, 708, 709, 809, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090
3	112–119, 122–129, 132–139, . . . , 192–199
	213–219, 223–229, 233–239, . . . , 293–299
	314–319, 324–329, 333–339, . . . , 349–399
	415–419, 425–429, 435–439, . . . , 495–499
	516–519, 526–529, 536–539, . . . , 596–599
	617–619, 627–629, 637–639, . . . , 697–699
	718, 719, 728, 729, 738, 739, . . . , 798, 799
	819, 829, 839, 849, 859, 869, 879, 889, 899
	1021–1029, 1031–1039, 1041–1049, . . . , 1091–1099
	2031–2039, 2041–2049, 2051–2059, . . . , 2091–2099
	3041–3049, 3051–3059, 3061–3069, . . . , 3091–3099
	4051–4059, 4061–4069, 4071–4079, 4081–4089, 4091–4099
	5061–5069, 5071–5079, 5081–5089, 5091–5099
	6071–6079, 6081–6089, 6091–6099
	7081–7089, 7091–7099
	8091–8099

[a] Not all sequential numbers after 99 are available (see text and Fig. 29C).

used, the same two-clip sequence is followed but the second toe on the left front foot is cut (LF2-RF1, LF2-RF2, . . ., LF2-RR5); this pattern is continued until all unique two-toe combinations are used (LR5-RR5). After all two-toe combinations are used, three digits are clipped in unique combinations. With this system, thousands of individuals can be marked by removing no more than two toes per foot. Waichman (1992) proposed an alphanumeric code that is equivalent to the code used by Donnelly (1989).

BRANDING

Four types of brands (tattoo, heat, silver nitrate, and freeze) have been used to mark amphibians. Kaplan (1958) tattooed frogs by scarifying a numeral into the ventral skin with a 27-gauge hypodermic needle. The needle did not penetrate the entire dermal layer. India ink mixed with a drop of glycerin was applied to the grooves to make the mark. Kaplan (1958) did not observe infection, redness (erythema) disappeared rapidly, and the numbers stood out vividly against the light ventral skin. Kaplan (1959) similarly marked frogs using a small electric tattoo maker with a narrow needle. He recommended tattooing the upper abdomen, after the skin was wiped dry with cotton. Most tattoo machines (Appendix 6) require electricity to power the needle. Two vendors (S & W Tattoo Supply Company and Tatoo-A-Pet) sell battery-operated machines for field use. These machines are portable and less expensive than those that require electricity.

Clark (1971) used a small-diameter chromium (20%) and nickel (80%) wire that was shaped into numerals or letters to brand *Bufo valliceps*. He heated the brands with a small propane torch and applied them to the ventral skin until the dermal layer was penetrated. A scab formed over each mark but disappeared after 2 weeks. Brands were legible for more than 20 months.

Thomas (1975) branded *Hyla cinerea* with silver nitrate. He made brands with commercial silver nitrate applicators (used in veterinary cauterization) and applied them to the dorsal skin. A brown mark formed on the skin immediately upon contact. One frog smeared silver nitrate over its dorsal surface; it was found dead 5 days later. Thomas noted that frogs had a brief reaction to the chemical, but he reported no other adverse effects. The dark mark faded 2 weeks after application.

Daugherty (1976) described freeze branding of *Ascaphus truei*. He fashioned branding irons from 12-cm lengths of insulated copper electrical wire. He removed the insulation from the terminal 3 cm of the wire and shaped it into numerals with pliers. He placed the branding irons in chips of dry ice (liquid nitrogen could also be used) for 30 minutes and then applied one to the ventral skin for approximately 10 seconds; the skin was not penetrated. The branding iron was returned to the dry ice and could be used again after 1 minute. Brands were legible within 24 hours and were visible for up to 2 years.

Marking larval amphibians

Marking larvae is difficult, and most methods generate date-specific rather than unique individual marks. Methods include fin clipping (Turner 1960; Guttman and Creasey 1973) and staining by immersion in a solution of neutral red dye (Herreid and Kinney 1966; Guttman and Creasey 1973). Travis (1981) found that the mark generated by the latter procedure was short-lived and resulted in decreased tadpole growth even at low tadpole densities. Seale and Boraas (1974) injected organic dyes into the tail; Cecil and Just (1976) injected acrylic polymers into the tail fins.

Ireland (1973) marked salamander larvae on the middorsal surface of the body with a paste made of fluorescent pigments and acetone. He applied the mark with a heated probe that had been dipped in the pigment paste. The probe burned through the outer epidermal layers, and the pigment was incorporated into the regenerated epithelium. Ireland (1983a,b; 1989) used two additional marking methods for salamander larvae. Small larvae (total length < 15 mm) were marked with a spray-paint gun that contained fluorescent pigment. Large larvae (total length > 15 mm) were tagged by applying a paste made of Congo red stain and dimethyl sulfoxide (DMSO) to a small lesion on the tail. He created the lesion by reflecting the skin on the lateral tail and teasing the superficial muscle with a sharp probe. After the paste was applied, the wound was sealed with super glue. The red stain was incorporated into the muscle after 15 days and was visible with a 10× hand lens.

Recommendations

Marking methods should be tested in the laboratory and in the field prior to the initiation of any long-term study. We do not recommend jaw-tagging amphibians, because tag loss violates mark-recapture assumptions 2 and 4 (see "Background" under "Mark-Recapture" in Chapter 8). Tags should not snag vegetation or interfere with movement or reproductive activities. Fluorescent marks and tags may make animals more conspicuous to investigators and predators. Because PIT tag implantation could damage internal organs, we recommend inserting PITs into the dorsal lymph sac rather than intraabdominally. Silver nitrate brands should be avoided because Thomas (1975) observed a reaction by the frogs to the chemical. Brands should never be applied to the ventral "pelvic patch," because that site is important in water-balance physiology of anurans. Branding methods should be practiced before adoption, so that damage to underlying muscles and organs can be avoided.

Pattern mapping does not affect animals adversely, but the methodology is relatively expensive if animals are photographed. Sketching is time-consuming but inexpensive and does not harm the study organisms; we recommend this method for species in which the variation in color pattern allows for individual recognition.

Although Clarke (1972) demonstrated that toe clipping can have adverse effects, it is the least expensive marking method that enables one to mark large numbers of animals quickly. This marking method may not be suitable for salamanders, because they regenerate toes quickly. Handling time in the field (especially for anurans) and equipment costs for toe clipping are minimal relative to time and costs required for other marking and tagging methods.