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Effects of Toe-clipping and PIT-tagging on Growth and Survival in Metamorphic *Ambystoma opacum*

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Long-term demographic studies of wild populations require the ability to mark individuals permanently (Ferner, 1979). For many studies, it is imperative that individuals within populations be censused repeatedly over time so that information on growth rates, immigration or dispersal patterns, and population density can be obtained (Jemison et al., 1995). Especially with endangered, threatened, or declining species, a marking system may be of limited or unknown usefulness because the risks of marking individuals have never been determined. Without the ability to monitor marked individuals over time, critical information on the life history and demography of a species is lost. This information may be essential for determining appropriate management alternatives for both abundant and recovering populations.

Ferner (1979) reviewed the common marking techniques used in studies of amphibians and reptiles. An ideal mark or tag should: (1) not affect the survivorship or behavior of the individual, (2) uniquely identify the individual, (3) be permanent over the individual's lifetime (4) be easily discernible, and (5) be easily implemented in both laboratory and field experiments (Ferner, 1979). In addition, use of a tag or mark should be cost-effective and should attempt to limit handling time of an individual.

Toe-clipping has been the most frequently employed method for marking amphibians (Ferner, 1979), and has been used for several decades in long-

term studies. Toes are removed with dissection scissors in specific combinations that correspond to a prescribed numbering system (Twitty, 1966). This method is cost-effective and minimizes handling time of animals. However, salamander toes often regenerate or are lost naturally, making individual codes difficult to discern over time (Ferner, 1979). Furthermore, the removal of toes may impede locomotion or reproduction in some amphibian species (Clarke, 1972; Camper and Dixon, 1988).

Recently, Passive Integrated Transponder (PIT) tags have been implemented as an alternative for marking animals. A PIT-tag is a small (12 × 2.1 mm) transponder hermetically sealed in biocompatible glass (AVID Marketing, Inc., Norco, CA). Each tag is encoded with a 10-space alpha-numeric code that is read with a handheld electromagnetic scanner. The scanner energizes the tag and transmits the individual code to a reader where it is displayed (see Camper and Dixon, 1988; Zulich et al., 1992 for further information). PIT-tags provide a clear and easily recognizable "mark" that is generally permanent over an individual's lifetime. In most cases, PIT-tags are inserted into the animal's body cavity, which may increase the handling time associated with marking the individual. PIT-tags have been used to mark a variety of species, including mammals (Schooley et al., 1993), commercial fish (McCutcheon et al., 1994; Peterson et al., 1994), snakes (Keck, 1994; Jemison et al., 1995), lizards (Germano and Williams, 1993), and frogs (Camper and Dixon, 1988).

Few studies have examined the effectiveness of using PIT-tags in salamanders (but see Faber, 1997). This study was designed to test the effects of toe-clipping and PIT-tagging on survival and growth in metamorphic *Ambystoma opacum*. In addition, because numerous long-term demographic studies of amphibians have been conducted at our laboratory (e.g., Pechmann et al., 1991; Semlitsch et al., 1996; Taylor and Scott, 1997), we evaluated the usefulness of each technique for large-scale field studies.

Study Area.—We conducted our study at Ginger's Bay, a temporary Carolina bay wetland (0.8 ha) located in the northwestern portion of the Savannah River Site, Aiken Co., SC (Scott, 1990). Carolina bays are shallow, upland wetlands of unknown origin located on the southeastern Atlantic Coastal Plain. Many Carolina bays are temporary, filling in late autumn and drying in early spring (Jackson et al., 1989), and provide important breeding sites for amphibians. We chose Ginger's Bay for our study because it supports a large breeding population of marbled salamanders, typically produces metamorphic juveniles each year that can be captured easily by a drift fence with pitfall buckets (Krenz and Scott, 1994), and contains intact hardwood habitat around the margin of the bay in which we could place terrestrial enclosures.

We placed four large (2 m diameter) and four small (1.5 m diameter) cattle tanks in the upland forest near the edge of the bay. Cattle tanks were set 17 cm into the ground to allow soil temperatures in the tanks to approximate actual soil temperatures. Each tank contained roughly 17 cm of soil and 5 cm of leaf litter raked from the terrestrial habitat near the bay. In addition, we placed three 30 × 61-cm pieces of particle board and four decaying logs and/or sections of pine

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bark under the leaf litter to provide adequate cover for the animals. We set small sections of 1.9-cm diameter PVC pipe under coverboards as artificial burrows and to elevate the boards so that salamanders could access the refuges more readily. Tanks were covered with hardware cloth fixed on a wooden frame to deter mammalian predators. Small tanks were numbered T1–T4 and large tanks T5–T8.

On 7 May 1996, we collected 260 newly metamorphosed *A. opacum* from pitfall buckets as animals migrated out from the bay. Salamanders were held in a walk-in cooler at 5 C for two weeks, until we were confident no other large migrations of *A. opacum* would leave the bay and we had adequately sampled the 1996 cohort. On 22 May 1996, we weighed each animal (± 0.01 g) and measured its snout-vent length (SVL; ± 0.5 mm). Animals were then housed overnight in individually marked plastic containers until assigned to treatments.

Salamanders were sorted by body mass into large ($N = 87$, $\bar{x} = 3.34 \pm 0.26$ g, range = 3.05–4.05 g), medium ($N = 86$, $\bar{x} = 2.89 \pm 0.09$, range = 2.73–3.05 g), and small ($N = 87$, $\bar{x} = 2.34 \pm 0.27$, range = 1.67–2.73 g) weight groups. Each weight group was then sorted into eight additional sub-groups based on mass to insure that the distribution of body size in each treatment group in all cattle tanks was approximately the same. We randomly assigned salamanders within these sub-groups to one of three treatments (control, PIT-tagged, and toe-clipped) or to a reserve group (to be used in the case of mortality during marking). Each tank therefore contained 10 animals from all three treatments, for a total of 30 animals per tank (control animals assigned to odd numbered tanks were "true" controls, and no technique was applied; those assigned to even numbered tanks were "sham-operated" controls). Animals not used in the study were released at Ginger's Bay.

Toe-clipped Group.—Salamanders were marked by removing two adjacent toes of one foot, using a numbering system similar to Twitty (1966). Each animal within a tank was given an individual mark by clipping toes in one of 10 unique combinations. The same set of 10 codes was repeated for each group of toe-clipped animals in all eight tanks.

PIT-tagged Group.—We used a surgical technique to implant PIT-tags rather than hypodermic injection. Animals were anesthetized using 2-phenoxy ethanol (30 drops/500 ml dH₂O) for about ten minutes. A 3-mm incision was made through the skin on the right side about 5 mm anterior to the hind limb, and the PIT-tag was inserted forward into the body cavity. New skin® liquid bandage was applied to the incision and allowed to dry. Animals were then placed in dH₂O with their heads above water until the anesthetizing agent wore off and salamanders appeared active when nudged. Recovery typically took 3–4 h, but often required more time before salamanders became fully active.

Control Groups.—Sham-operated controls received the same treatment as PIT-tagged animals except that tags were immediately removed from the animal's body after insertion. Animals were not permanently marked, although the incision left a discernible scar for several months. True control animals were not marked in any way.

All toe-clipped animals were marked on 25 May 1996. PIT-tagged animals in tanks 1, 2, 4, and 6–8 were marked on 25 May, and animals in tanks 3 and 5 were marked on 26 May. All sham operations were conducted on 26 May. Two animals in the PIT-tagged treatment died during the recovery period and were replaced with two individuals of similar body size from the reserve group. Animals were held for observation one night following marking, and then were released the following day into assigned terrestrial cattle tanks. Tanks were sprayed with dH₂O on 27 May to simulate a rainfall event and were watered throughout the study when the soil became dry to the touch. Each salamander had available about four crickets (125 per tank) dusted with Rep-Cal® ultrafine calcium powder and Herptivite® vitamin supplement per week.

Tanks were censused bimonthly until November, starting on 11 June. Toe-clipped animals were identified by reading individual toe-clip marks. PIT-tagged animals were distinguished from control and sham-operated animals by passing the scanner over each animal and reading the ensuing code if the animal had a PIT-tag. Animals without tags or toe-clips were recorded as control animals; control groups in some tanks contained more than ten individuals because some animals in the PIT-tagged treatment lost their tags. However, these previously PIT-tagged animals could generally be distinguished from true controls by the presence of scar tissue, although their individual ID was no longer known. Salamanders were removed from tanks on 17 July, 10 September, and 11 November and brought to the lab where they were weighed and measured.

Statistical Analysis.—Untransformed data from the initial (May) and final (November) sample dates were analyzed separately. Analysis of variance (ANOVA) was used to test the effects of marking treatment on body mass, SVL, and number of surviving animals; survivorship was estimated as the minimum number alive (MNA) in September. Individuals in the PIT-tagged group that lost PIT-tags were removed from the data base prior to statistical analyses. Tank means for each treatment group were used as the unit of observation because individuals within a tank are not independent observations (Hurlbert, 1984). The TREATMENT*TANK term in a Type III SS ANOVA model was used to test for treatment main effects. Analyses were conducted using the General Linear Models procedure of the Statistical Analysis System (SAS Institute, 1994).

Sham vs. True Controls.—Sham-operated animals and true-control animals did not differ in body size at the start of our study (SVL: $F_{1,6} = 3.06$, $P = 0.13$; Mass: $F_{1,6} = 3.44$, $P = 0.11$). By November, there were still no detectable body size differences between the two control treatments (SVL: $F_{1,6} = 0.09$, $P = 0.77$; Mass: $F_{1,6} = 0.02$, $P = 0.90$). Therefore, the control groups were combined into a single control treatment.

Treatment Comparison.—No differences were observed among individual tanks or among treatment groups (PIT-tag, toe-clip, and control) in mean body size at the start of the experiment (SVL: $F_{2,21} = 0.12$, $P = 0.89$; Mass: $F_{2,21} = 0.05$, $P = 0.95$; Fig. 1). Although animals in some tanks grew significantly more slowly than those in other tanks, similar reductions in

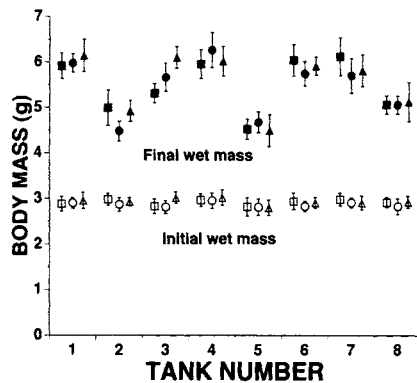


FIG. 1. Initial and final body mass means (± 1 SE) for each treatment group (open symbols = initial, solid = final; squares = controls, circles = PIT-tagged, triangles = toe-clipped) in the eight experimental tank enclosures.

growth occurred in all treatments; we found no differences among treatments in the November sample (SVL: $F_{2,21} = 0.52$, $P = 0.60$; Mass: $F_{2,21} = 0.05$, $P = 0.95$; Fig. 1). Based on the MNA estimates for September, all groups had 92–98% survivorship (arcsine-square-root transformed survival: $F_{2,21} = 1.38$, $P = 0.27$; Fig. 2).

We found no significant short-term effect of either toe-clipping or PIT-tagging on growth or survival in *A. opacum*. These results suggest that both techniques may be effective for marking salamanders. However, if we re-examine the list of criteria for an ideal tag or mark provided in Ferner's (1979) review, neither toe-clipping nor PIT-tagging satisfy all of the criteria for *A. opacum*. Because a marking technique that satisfies all criteria is currently not available for salamanders, the usefulness of available techniques in field studies must be considered.

Over the past few decades, toe-clipping has been the most common technique used for marking salamanders, primarily because it is easily implemented in field studies, inexpensive, and relatively risk-free to animals. In this study, we observed no effects of clipping two toes per animal compared to control animals. In addition, individual codes can be issued in unique combinations and are usually easily read by investigators. However, because salamanders regenerate cut toes and sometimes suffer toe injury, the identity of an individual over time may become ambiguous. In some species, complete regeneration may take several years (Ferner, 1979), and toes can be re-clipped before an individual mark is no longer discernible. However, in many salamander species, toes can completely regenerate before the animal is observed again (Ferner, 1979), making identification difficult. For example, in our experience with field studies of *A. opacum*, "group" codes (cutting two adjacent toes, as described here) are generally retained well and are recognizable years later, whereas "individual" codes (1–2 toes on 1, 2, 3, or 4 feet) may be difficult to interpret two or three years later. A closely related species, *Ambystoma talpoideum*, matures at an earlier age than *A. opacum* and seems to retain marks better,

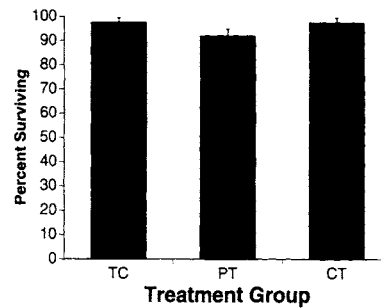


FIG. 2. Treatment group (toe-clipped, PIT-tagged, and control) means (± 1 SE) for Minimum Number Alive (MNA) percent survival estimates in September 1996.

making it a better candidate for individual marks (see Semlitsch et al., 1988). Therefore, an analysis of the regeneration time and extent for each species must be considered before toe-clipping can be used effectively in large, long-term field studies. Toe regeneration can be reduced by applying compounds such as beryllium nitrate (Heatwole, 1961), but this treatment increases the cost and handling time associated with marking an individual.

PIT-tagging offers a clear, easily readable code, making the identification of a recaptured individual unambiguous. In our study, we experienced a problem in six of the experimental enclosures, where two to five ($\bar{x} = 2.25$) PIT-tagged animals lost tags within the first two weeks of the experiment. Furthermore, two PIT-tagged salamanders died during the initial marking period. Our assessment, however, is that tag loss and immediate mortality due to surgery are not inherent problems of the PIT-tagging technique, but were due to our inexperience in implanting the tags. A high incidence of tag loss in a population study would clearly be unacceptable because a lost tag in a mark-recapture study is interpreted as mortality or emigration. We are confident that the technique itself, once practiced, would incur minimal tag loss.

Newly metamorphosed *A. opacum* are relatively small, have flexible bodies, and have skin that tears fairly easily. For these reasons, we decided not to inject PIT-tags into the body cavity with a 12-gauge needle and syringe as is typical for larger species (e.g., snakes, mammals, fish). Instead, we surgically implanted the tags, which required anesthetizing animals and increased the time needed to mark each salamander. Recovery time for salamanders ranged from three to eight hours, greatly increasing the amount of time the salamanders remained in the lab. The extended recovery period may be due to the anesthetic (2-phenoxy ethanol) we used; other options (e.g., MS-222; see Faber, 1997) could decrease the recovery time substantially. Nonetheless, PIT-tagging does not seem a practical method for large-scale salamander studies. Due to the episodic nature of adult breeding migrations and juvenile emigrations, minimizing handling time is an essential component of mark-recapture protocol. At study sites where *Ambystoma* are common, we routinely capture thousands of adults and up to 10,000 newly metamorphosed juveniles in a single

night. Whereas two researchers can toe-clip about 4000 animals in eight hours in the field, we believe far fewer animals could be PIT-tagged during the same amount of time. PIT-tagging becomes a much more viable option if tags can be injected and not surgically implanted. Although we feel that 1–3 g salamanders were too small for injection, larger animals (>10 g) could have tags injected, thereby reducing handling time. The size range of animals used in this study did not allow us to assess the effects of injecting the tag. If we assume that the injection itself would not imperil larger animals, then the placement of PIT-tags in the coelomic cavity should not be detrimental to growth and survival (as we demonstrated in this study).

Faber (1997) PIT-tagged 365 adult newts (*Triturus alpestris*; SVL > 40 mm), captured over a 2-mo period, and examined movement among 17 breeding ponds by males and females. By using an underwater extension attached to the PIT-tag scanner Faber was able to locate newts in the ponds at distances up to 15 cm. The recapture rate of PIT-tagged females (82.2%) was significantly greater than the rate for males (74.6%), which Faber believed was due to a greater inter-pond migration frequency in males (37.7%) than in females (23%). For a study with the objectives of Faber's, PIT-tags provided the advantages of unique individual identification, reducing handling time for recaptured animals, and locating animals under vegetation and pond substrate. Thus, in studies of limited aspects of the population ecology of adult salamanders (*sensu* Faber, 1997) PIT-tagging may be effective because recapture rates can be high and overall handling time may actually be reduced.

An additional concern of the PIT-tagging technique is cost; it is an expensive method to implement (Faber, 1997). In 1996, each tag ranged in cost from \$4.75 to \$6.00, depending on the quantity of tags ordered, and the least expensive reader available was about \$500.00 (AVID Marketing, Inc., Norco, CA). Faber (1997) stated that the PIT-tag method would nonetheless be useful for studies of population dynamics. However, recruitment of newly metamorphosed juveniles into the terrestrial population is presumably an integral component of the dynamics, yet numerous small metamorphs are ill-suited for PIT-tags. For example, at one of our study sites (Rainbow Bay) in 1984 over 19,000 juvenile red-spotted newts (*Notophthalmus viridescens*; SVL = 23.1 ± 0.1 mm) were captured leaving the pond (Semlitsch et al., 1996). Additionally, PIT-tagging may not be the most cost-effective method because it is likely that many of the marked animals will not be recaptured in subsequent years, especially if initial marking is of recent metamorphs. The proportion of metamorphs returning as adults averages <20% for *A. opacum* (Scott, 1994), and approximately 25% for *A. talpoideum* (Semlitsch et al., 1988). For a cohort of 10,000 juveniles, most research projects cannot afford to spend \$40,000 marking animals that may never be seen again.

We are left with the dilemma that the "ideal" marking technique for long-term field studies of salamanders remains elusive. Ideally, each individual receives a unique ID code and is followed unobtrusively and non-destructively throughout its lifetime. Repeated captures of individuals provide the necessary data for individual-based allocation, population, and life-his-

tory models. However, for the present we suggest a combination of existing techniques that will provide nearly ideal data in population studies of post-metamorphic, pond-breeding salamanders. Two adjacent toes can be clipped on a single foot in recently metamorphosed juveniles; 14 combinations are possible for most salamanders. Adjacent toes are recommended because the mark is generally recognizable in subsequent years even in species that regenerate toes. Because size at metamorphosis is related to adult traits that are correlated with fitness (Semlitsch et al., 1988; Scott, 1994), the investigator(s) may choose to divide the cohort into body size categories, with each size class receiving a distinctive mark. However, if the study of a particular population will continue for many years, group marks should be used judiciously; 14 combinations can be used quickly. By using primarily the toe-clipping technique for juveniles, numerous animals can be marked quickly and cheaply in the field in minimal time. If the investigator chooses to PIT-tag a subset of the newly metamorphosed cohort, those animals should also receive a unique toe-clip mark to externally identify them as "PIT-tagged." When adults return to the breeding site, animals from the group-marked cohort of metamorphs can be individually PIT-tagged or toe-clipped (if regeneration is not a concern for the species). Individual juvenile growth data would be missing for cohort-marked animals, but the individual data from the subset of PIT-tagged metamorphs may alleviate this problem somewhat.

Although we have not discussed pattern mapping (Ferner, 1979), photos of unique patterns may also be used to identify individuals of some species. For the ambystomatid species with which we are familiar, using patterns is not possible for unpatterned juveniles and is cumbersome when adult population sizes number in the thousands. Of the current marking techniques available, toe-clipping remains the most economical and practical method for studies of pond-breeding salamanders. An alternative marking technique that incorporates more of the criteria given by Ferner (1979) will be essential for future long-term studies.

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Courtship Behavior in a Polymorphic Population of the Tiger Salamander, *Ambystoma tigrinum nebulosum*

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In some salamander species, larvae either transform into terrestrial metamorphic adults or mature while remaining in an aquatic larval form to become paedomorphic adults, a polymorphism termed facultative paedomorphosis (Semlitsch, 1987; Whiteman, 1994). The expression of each developmental pathway depends on a variety of proximate environmental conditions as well as genetic factors (Collins, 1981; Harris, 1987; Semlitsch and Wilbur, 1989; Semlitsch et al., 1990). However, few studies have concentrated on the fitness consequences of each morph (Whiteman, 1994, 1997; Ryan and Semlitsch, unpubl. data).

Natural selection would operate on this polymorphism if the environment of one morph becomes harsh and causes differential mortality of adults (e.g., pond drying or freezing) or decreases reproductive output (e.g., due to increased competition for resources). Sexual selection would operate if morphs have differential reproductive success during mating bouts. For example, males of one morph might outcompete males of the other morph for mates, or be preferentially chosen by females (Whiteman, 1994).

Facultative paedomorphosis is environmentally induced and occurs in both sexes; thus, natural selection

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