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Effects of Toe-Clipping on the Survival and Growth of *Hyla squirella*

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Toe-clipping is a widely used and cost-effective method for marking amphibians for ecological studies, but recent research has suggested that toe-clipping may cause adverse impacts on amphibian health and behavior. Several studies have documented decreased growth or recapture rates in toe-clipped animals (Davis and Ovaska 2001; McCarthy and Parris 2004). Other studies have noted inflammation and necrosis of clipped toes, suggesting this method may adversely affect mobility and survivorship (Davis and Ovaska 2001; Golay and Durrer 1994; Lemckert 1996). Recently, McCarthy and Parris (2004) questioned the ethics of toe-clipping based on findings that recapture rates in four studies decreased linearly with the number of toes removed.

One important assumption of mark-recapture studies is the mark has no influence on the survival or recapture probability of an animal (Donnelly and Guyer 1994). Thus, any direct or indirect impact of toe-clipping, such as negative effects on growth, health, or behavior may bias results unless those effects are known and can be accounted for during data analysis (Donnelly and Guyer 1994). Unfortunately, study results conflict over the existence and degree of such effects. Instances where toe-clipping did not affect survivorship or growth were documented by Ott and Scott (1999) for *Ambystoma opacum* and by Van Gelder and Strijbosch (1996) for *Bufo bufo*. However, the magnitude and cause of adverse impacts are difficult to quantify in the field and have not conclusively been addressed under controlled settings.

In a laboratory study, we evaluated the impact of toe-clipping on growth and survivorship of Squirrel Treefrogs (*Hyla squirella*). Conducting the study in a controlled setting allowed us to separate direct effects of toe removal on survivorship and growth due to physiological stress and wound infection, from possible indirect effects such as behavioral avoidance of capture locations and decreased foraging success or increased predation due to impaired mobility.

Methods.—Sixty-two adult *H. squirella* were collected in Baker County, Georgia, USA (31°13'16.88"N, 84°28'37.81"W) during November 2004 and maintained in the lab until sufficient numbers were gathered for the study. On 2 December 2004, frogs were weighed (± 0.01 g) and the snout-vent length (SVL; ± 0.1 cm) was

measured. To ensure that size at capture did not bias the results, frogs were sorted by body mass into small (N = 22, mean = 1.44 g, range = 1.10–1.86 g), medium (N = 20, mean = 2.22 g, range = 2.01–2.53 g) and large (N = 20, mean = 2.82 g, range = 2.59–3.21 g) size groups, and individuals within each size group were randomly assigned to one of two treatments (control or toe-clipped) using PROC SURVEYSELECT in SAS version 8.2 (SAS Institute Inc. 2001, Cary, North Carolina, USA). Each size group was equally represented in each treatment.

Animals in the toe-clipped treatment were marked by complete excision of the third toe on the left front foot and the fourth toe on the right rear foot with scissors sterilized in 95% ethanol. This mark corresponded to a mark of 72 under the Hero (1989) marking scheme. All wounds from toe removals were sprayed immediately with Bactine®, as recommended by Martin and Hong (1991). Animals in the control group were not toe-clipped or marked in any way. All animals were housed individually in 15 × 15 × 6 cm plastic Tupperware® containers on a substrate of moist paper towels. Frogs within the same size group were offered the same number and size of crickets 2–3 times a week. Crickets were dusted biweekly with Herpcare Cricket Dust®, which served as a vitamin supplement. All containers were maintained at a temperature of ca. 20ºC, and a 12:12 L: D photoperiod was maintained with full spectrum 60-watt incandescent bulbs hung 1 m above the containers. Individuals from each treatment were measured and weighed and their toes were examined monthly for signs of inflammation for five months. Survivorship of frogs from each group was also recorded. The sex of each frog was determined at the end of the study when secondary sexual characteristics (distended vocal sacs in males) were expressed with the onset of the breeding season.

Repeated measures analysis of variance was used to compare changes in SVL and mass among treatment groups using size group as a blocking variable. To test for any influence of sex on growth response variables, a separate repeated measures analysis of variance was used to compare SVL and mass among the sexes with size group as a blocking variable. Frogs with missing data (two escapes, and one death) were not included in the analysis. Analyses were conducted using the GLM procedure with a repeated statement in SAS version 8.2 (SAS Institute Inc. 2001, Cary, North Carolina, USA). This procedure produces both univariate and multivariate (Manova) tests for the effects of the treatments and time on the response variable.

Results.—One toe-clipped animal died of unknown causes during the fourth month, and two control animals escaped. Inflammation of clipped toes was recorded in nine animals (29%) during the first month and in two animals (6.5%) during the second month of the experiment. However, the wounds of all animals healed after 2 months, and no instances of tissue necrosis were observed.

Mean mass (t_{57} = -0.12, p = 0.91) and SVL (t_{57} = 0.28, p = 0.78) did not differ between treatment groups at the start of the experiment. Treatment had no effect on SVL ($F_{1, 55}$ = 0.00, p = 0.95) or mass ($F_{1, 55}$ = 0.10, p = 0.75), and there was no treatment by time interaction for either response variable (in both univariate and multivariate tests) (Figs. 1 and 2). However, both mass and SVL varied over time (mass: Wilk's lambda = 0.16, $F_{5, 51}$ = 52.63, p < 0.0001, univariate $F_{5, 275}$ = 40.91, G-G adjusted p < 0.0001; SVL: Wilk's lambda = 0.33, $F_{5, 51}$ = 20.60, p < 0.0001, univariate $F_{5, 275}$ = 29.07, G-G adjusted p < 0.0001). Neither SVL nor mass was significantly different between the sexes (SVL: $F_{1, 55}$ = 0.16, p = 0.69; Mass: $F_{1, 55}$ = 0.00, p = 0.98), and the sex by time interaction was not significant in any test.

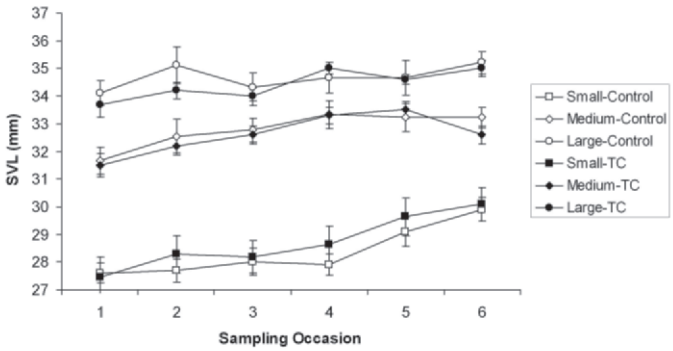

FIG. 1. Mean (± SE) SVL of toe-clipped (TC) and control frogs within different size groups sampled at 1 month intervals from December 2004 – May 2005. The 1 mm decrease between sampling events 5 and 6 for the medium sized toe-clipped group was due to measurement error.

Discussion.—Toe-clipping did not significantly affect the short-term growth or survivorship of *H. squirella* in this experiment. Our results are similar to those of Davis and Ovaska (2001) who examined the survivorship and growth of toe-clipped *Plethodon vehiculum* under laboratory conditions and to those of Ott and Scott (1999) who found no effects of toe-clipping on *Ambystoma opacum* maintained in small outdoor enclosures. Van Gelder and Strijbosch (1996) similarly found no differences in food consumption or mass between toe-clipped and unmarked *Bufo bufo* kept in small outdoor vivaria. Taken together these studies provide no evidence for a direct negative effect of toe-clipping on the health and growth of amphibians when they are provided ample food and maintained in a controlled, predator-free environment.

However, several studies conducted in field situations have demonstrated that amphibians marked by toe-clipping are recaptured less often than individuals marked by other methods, and that return rates are negatively related to the number of toes removed (Davis and Ovaska 2001; McCarthy and Parris 2004). In light of the laboratory studies, these observations suggest two possible

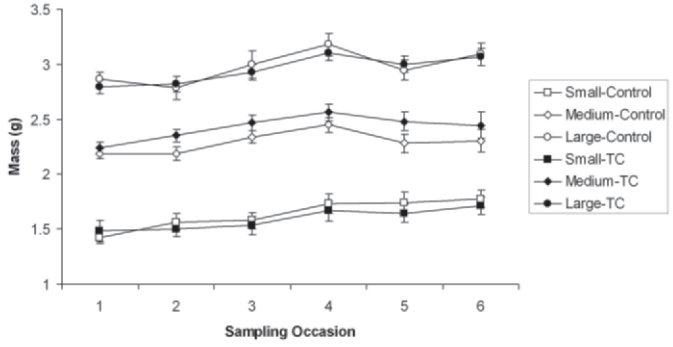

FIG. 2. Mean (± SE) mass of toe-clipped (TC) and control frogs within different size groups sampled at 1 month intervals from December 2004 – May 2005.

hypotheses: 1) toe-clipping may have an indirect negative effect on survival through increased predation and/or decreased foraging ability due to decreased mobility, 2) toe-clipping may affect amphibian behavior causing individuals to avoid capture locations. Evidence exists for both hypotheses. In a field experiment, Davis and Ovaska (2001) found that daily weight gain of *P. vehiculum* was significantly lower in toe-clipped animals than in unmarked individuals. However, they noted that both marked and unmarked *P. vehiculum* were consistently found under the same cover objects where they were initially captured. In contrast, Castellano and Giacoma (1993) found a negative correlation between the number of toes clipped and the number of days male *Bufo bufo* spent at a breeding pond. Similarly, Lemckert (1996) noted that 25% of newly toe-clipped *Crinia signifera* caught immigrating into a breeding pond left the pond within 1–3 days of marking, a behavior never observed in returning, previously marked individuals.

One consideration when gauging the impacts of toe-clipping is that individual species may be affected to different degrees and through varying combinations of mechanisms. Species with different feeding strategies, habits (e.g., arboreal vs. fossorial), antipredator behaviors, or habitat relationships may be differentially affected by the removal of one or more toes. Species also may vary in degree of susceptibility to stress or infection. For example, Lemckert (1996) found only rare incidences of infection in toe-clipped *C. signifera* but an almost 100 % infection rate in *Uperoleia laevigata*. Thus, marking by toe-clipping may be an effective, nondisruptive technique for some species but not for others.

The ethics of toe-clipping were recently questioned when McCarthy and Parris (2004) found that return rates of several frog species decreased linearly with the number of toes removed. Their study provides clear evidence that toe-clipping induces a negative effect on either the survivorship or the behavior of frogs. However, all available marking methods for amphibians involve the handling of animals and/or invasive procedures (see Donnelly et al. 1994), and it is likely that all marking methods induce some negative effect on the marked animals. In order to assess the ethics of toe-clipping and to determine the least disruptive marking method, the nature of the negative effects of toe removal should be fully understood. For instance, a marking method that results in a decrease in return rates due to behavioral avoidance of the capture site would be preferable to a method that results in a decrease due to mortality, particularly in the case of a rare or protected species. Unfortunately, few studies have compared the effects of toe-clipping to other marking methods (see Davis and Ovaska 2001) and the effects of other marking procedures on amphibian health and behavior are poorly known.

It should be emphasized that this study had several limitations, which should be carefully considered when interpreting the relevance of our results to the practice of toe-clipping in field studies. First and foremost, this was a laboratory study in which frogs were maintained in small enclosures in a predator-free, constant environment with an abundant and easily accessible food supply. This study could therefore not measure negative effects on the climbing ability and general mobility of the treefrog *Hyla squirella* or assess any influence of toe removal on behavior. However, given the importance of the adhesive toe discs of treefrogs in climbing and movement, it is likely that toe-clipping does have a negative effect on the mobility of this species. In addition, it is possible that our study animals healed at a different rate than they would have in the wild where a wider range of thermal environments, food items, and opportunities for infection exist. Secondly, all excision wounds in this experiment were treated with antiseptic, which may not be a common practice in most field studies. Our results may therefore represent an underestimate of the negative impacts of toe-clipping on growth and survivorship compared to field studies where antiseptic is not used. However, despite the limitations of this study, we believe our results add to a growing body of evidence that suggests that the negative impacts of toe-clipping observed in field studies are related to factors other than direct health effects.

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Literature Cited

Castellano, S., and C. Giacoma. 1993. A comparison of individual marking techniques applied to the study of wild populations of *Bufo bufo*. Suppl. Ric. Biol. Selvaggina 21:685–692.

Davis, T. M., and K. Ovaska. 2001. Individual recognition of amphibians: Effects of toe clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. J. Herpetol. 35:217–225.

Donnelly, M. A., and C. Guyer. 1994. Estimating population size. *In* W. R. Heyer, M.A. Donnelly, R. W. McDiarmid, L.-A. C. Hayek, and M. S. Foster (eds.), Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians, pp. 277–284. Smithsonian Institution Press, Washington D.C.

———, ———, J. E. Juterbock, and R. A. Alford. 1994. Techniques for marking amphibians. *In* W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L.-A. C. Hayek, and M. S. Foster (eds.), Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians, pp. 277–284. Smithsonian Institution Press, Washington D.C.

Golay, N., and H. Durrer. 1994. Inflammation due to toe-clipping in natterjack toads (*Bufo calamita*). Amphibia-Reptilia 15:81–96.

Hero, J. 1989. A simple code for toe clipping anurans. Herpetol. Rev. 20:66–67.

Lemckert, F. 1996. Effects of toe-clipping on the survival and behaviour of the Australian frog *Crinia signifera*. Amphibia-Reptilia 17:287–290.

Martin, D. and H. Hong. 1991. The use of Bactine® in the treatment of open wounds and other lesions in captive anurans. Herpetol. Rev. 22:21.

McCarthy, M. A., and K. M. Parris. 2004. Clarifying the effect of toe-clipping on frogs with Bayesian statistics. J. App. Ecol. 41:780–786.

Ott, J. A., and D. E. Scott. 1999. Effects of toe-clipping and PIT-tagging on growth and survival in metamorphic *Ambystoma opacum*. J. Herpetol. 33:344–348.

Van Gelder, J. J., and H. Strijbosch. 1996. Marking amphibians: effects of toe-clipping on *Bufo bufo* (Anura: Bufonidae). Amphibia-Reptilia 17:169–174.