



Management and Conservation

# Effects of Tail-Clipping on Survivorship and Growth of Larval Salamanders

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**ABSTRACT** Tissue samples used for genetic analyses are increasingly necessary for proper management of rare or endangered species, yet growing evidence suggests that traditional methods used to sample or mark amphibians have detrimental fitness effects. We used a semi-natural mesocosm experiment to determine the effect of larval tail-clipping on growth and survival of the endangered California tiger salamander. Even with relatively extreme levels of tail loss, we found no effect on survival, mass, or snout-vent length. We recommend larval tail-clipping as a low-impact method for collecting tissue samples from pond-breeding amphibians. © 2013 The Wildlife Society.

**KEY WORDS** *Ambystoma californiense*, cattle tank, genetic sampling, mark-recapture, regeneration, visual implant elastomer.

Field studies performed on living animals sometimes require invasive procedures, generally either to physically mark individuals (e.g., toe-clipping, ear-notching) or to obtain blood or tissue samples for genetic analyses. Criticism for the use of these marking techniques, particularly for amphibians and reptiles, has grown markedly since McCarthy and Parris (2004) showed that recapture rates of frogs decrease by 4–11% for each toe removed. A similar detrimental effect has also been found in salamanders, which are either less likely to be recaptured or have a decreased growth rate when toes are removed (Davis and Ovaska 2001, McCarthy et al. 2009). However, other studies have found no effect of toe-clipping in frogs (Lüddecke and Amézquita 1999), salamanders (Ott and Scott 1999), lizards (Borges-Landáez and Shine 2003), crocodylians (Jennings et al. 1991), or mammals (Fisher and Blomberg 2009). Debate over the use of toe clips is ongoing and includes an ethical dispute in which some have referred to the practice as “casual barbarity” (May 2004), whereas others have pointed out how “the resulting data are essential for managing threatened populations” (Funk et al. 2005).

One alternative method of collecting genetic samples from some amphibians that has been supported on ethical grounds is larval tail-clipping. The central argument is that larval amphibians have naturally high mortality rates; thus, if tail-clipped individuals do suffer reduced fitness it will still have a low impact on population growth rate (Parris et al. 2010). Whether this is the case depends on the

particulars of larval population biology, but it ignores the empirical question of whether tail-clipping actually decreases survivorship. A study on tail-clipping in adult newts found no effect of tail-clipping on either recapture rate or body condition and a potentially positive effect on snout-vent length (Arntzen et al. 1999). The only study that we know of that evaluated tail-clipping in larval salamanders used individually housed laboratory animals and thus could not evaluate the potential effect of competition or predation on growth and survival of clipped versus non-clipped individuals (Vaglia et al. 1997). It also used *Hemidactylum scutatum*, a species with a very truncated (20–40 days) larval period (O’Laughlin and Harris 2000). Such a truncated larval period could confound our understanding of the effects of tail-clipping because salamanders with short larval periods rely on their tails for aquatic foraging and predator evasion for a shortened period of time. Still, this study again found no effect of tail-clipping on snout-vent length at metamorphosis.

The California tiger salamander (*Ambystoma californiense*) is a listed species under both United States and California Endangered Species Acts, and is estimated to have disappeared from 34% of its original range (Davidson et al. 2002), although additional losses have occurred in the intervening decade. Historically, the greatest cause of decline has been habitat loss (Davidson et al. 2002). More recently, populations have been threatened by competition from and hybridization with invasive barred tiger salamanders (*A. mavortium*) introduced into their California range in the 1950s (Riley et al. 2003). Two important aspects of current and future conservation efforts on the California tiger salamander are the identification of genetic management

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units (Shaffer et al. 2004) and detailed genetic analyses of non-native barred tiger salamander genes moving across natural landscapes (Fitzpatrick and Shaffer 2007, Fitzpatrick et al. 2010). A key element of this work is the evaluation of genetic samples, which are virtually always taken as larval tail clips. Current federal regulations for California tiger salamanders state that researchers are allowed to remove at most the terminal 6.35 mm of tail (United States Fish and Wildlife Service, personal communication). Researchers have assumed that these alterations do not adversely affect survivorship of larval California tiger salamanders, although this has never been empirically evaluated. In addition, if larval California tiger salamanders are found to be unaffected by tail clips of 6.35 mm and larger, this information would be a boon to researchers who use those tail clips for genetic research. Larger samples could be used to perform additional runs of sequencing processes and unused tissue could be stored for future use.

The California tiger salamander is a large, robust salamander endemic to California that breeds primarily in fish-free vernal pools and cattle ponds (Trenham et al. 2000). Larvae feed upon aquatic invertebrates and larval amphibians (Anderson 1968, Ryan et al. 2009), sometimes including smaller conspecifics (H. B. Shaffer and C. A. Searcy, University of California, unpublished data). Larvae grow rapidly until metamorphosis, and recent field studies indicate that, as in many amphibians, a positive correlation exists between size at metamorphosis and post-metamorphic fitness (C. A. Searcy, unpublished data). Thus, a clear understanding of the quantitative relationship between larval tail removal and both survivorship and size at metamorphosis would form a valuable guide to current and future genetic sampling strategies. We conducted a semi-natural mesocosm experiment to evaluate the effects of tail-clipping on larval growth and survival in the California tiger salamander.

## METHODS

The salamanders for this experiment, conducted in spring 2012, came from 2 different laboratory crosses of pure California tiger salamanders, 1 from our long-term breeding colony, and 1 from a recently wild-caught pair of adults from the Jepson Prairie Preserve (Solano County, California). We placed adults in outdoor 1,136-L polyethylene cattle tanks on the University of California, Davis campus and allowed them to breed naturally. We harvested eggs after several days, randomly distributed them into batches of 15, and placed them in 4-L plastic shoeboxes in 10% modified Holtfretter's solution. We fed hatchlings brine shrimp (*Artemia salina*) nauplii daily (ad libitum) for about 7 weeks until they reached a size of  $47.2 \pm 6.5$  mm total length (all measurements are written as mean  $\pm$  SD).

We randomly selected 160 experimental larvae and divided them into 4 groups of 40 individuals. We designated 1 of these groups as the control, whereas the others received a small (2.5-mm), medium (5-mm), or large (10-mm) tail clip. We measured the size of the clip with a ruler with the larvae in the hand, as is often done in the field. On the following day, each larva received a treatment mark, which consisted of

a single subcutaneous injection of visual implant elastomer (VIE) in the left dorsum. Over the next 24 hours, we transferred the larvae to 1,136-L cattle tanks on the University of California, Davis campus. We transferred larvae to plastic bags, floated them in their experimental tank, and left them for 1 hour to equilibrate the water temperature in the bag with that in the tanks. We then changed 50% of the water in the bag and left the larvae in the bag overnight before we released them into the tanks the following morning. Ten replicate tanks each received 16 larvae (4 from each treatment). We stratified the larvae in each treatment between the 2 crosses (1 or 2 from the wild-caught cross and 2 or 3 from the colony cross; see Fig. 1 for representation of experimental design). Sixteen larvae per tank ( $6.4/m^2$ ) falls at approximately the 80th percentile of natural vernal pool densities (C. A. Searcy and H. B. Shaffer, unpublished data).

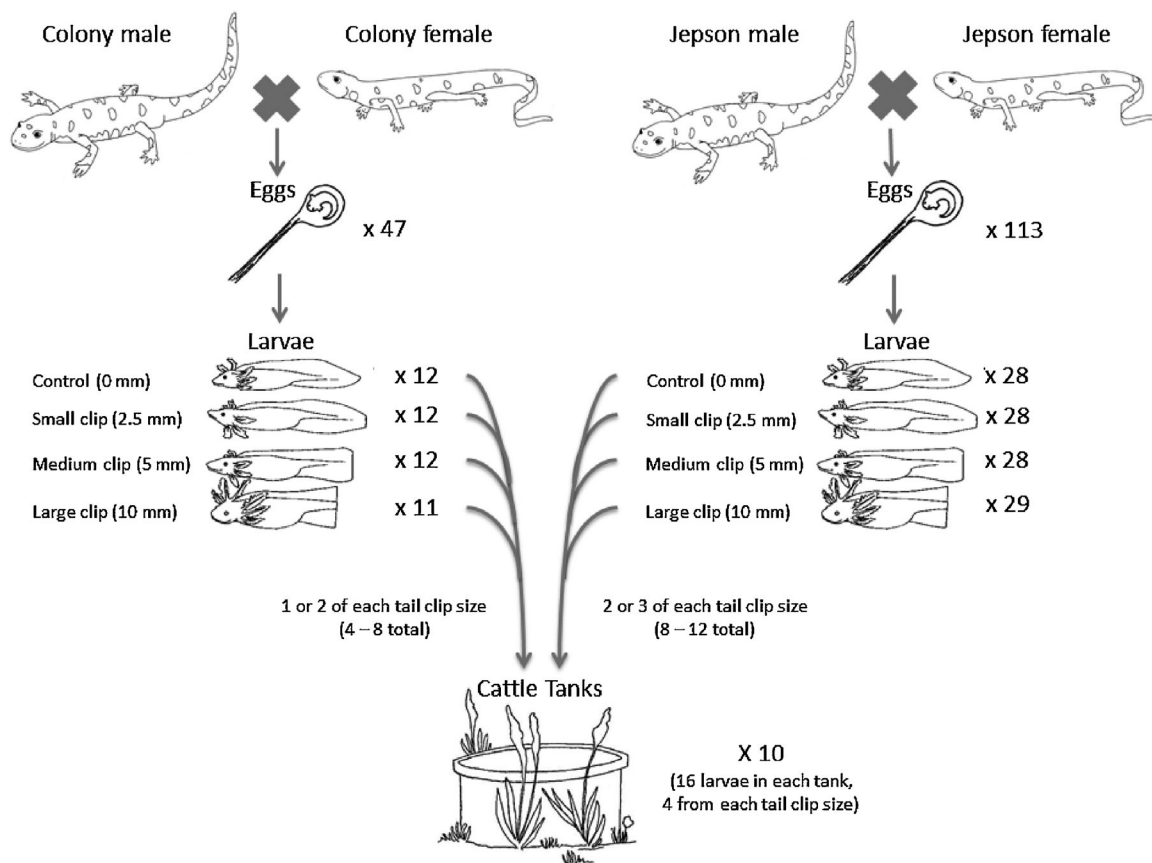
Before adding the larvae, we put 15.9 kg of rinsed Monterey beach sand in each tank as a natural substrate. We also covered each tank with a window screen mesh to exclude insect and vertebrate predators and the accumulation of natural debris. After we released the salamanders into the tanks, we fed them ad libitum with California black worms (*Lumbriculus variegatus*) twice per week. We kept the water in the tanks at a constant depth of 50 cm throughout the experiment and topped it off once per week.

We collected salamanders from the cattle tanks shortly before the most developmentally advanced larvae completed metamorphosis. We measured the mass, total length, and snout-vent length of every individual. We then euthanized the salamanders and dissected the VIE mark from their dorsum.

We used contingency tables to test for a treatment effect on larval survival. First, we tested for equal survivorship across all 4 treatments ( $2 \times 4$  contingency table, with up to 40 individuals per cell). As a second test, we pooled all 3 of the tail-clipping treatments and compared their survivorship to that of the control treatment using a  $2 \times 2$  contingency table. We tested for a treatment effect on larval growth rate using a separate 1-way analysis of variance (ANOVA) for each variable (mass, snout-vent length, and total length). In each of these ANOVAs, we blocked by tank to account for non-independence of larvae reared in the same cattle tank. We carried out all statistical analyses in R 2.13.1 (R Development Core Team, Vienna, Austria). We conducted salamander rearing and all experimental procedures under University of California, Davis IACUC protocols 16,206 and 16,345, United States Fish and Wildlife permit TE-094642-8, and California Department of Fish and Wildlife permits SC-2480 and SC-8437.

## RESULTS

Of the 40 salamanders that were originally in each treatment, 37, 38, 39, and 37 survived from the control, small clip, medium clip, and large clip treatments, respectively. We found no significant effect of treatment on survivorship regardless of whether all 4 treatments were compared in a  $2 \times 4$  contingency table ( $P = 0.998$ ) or whether the control



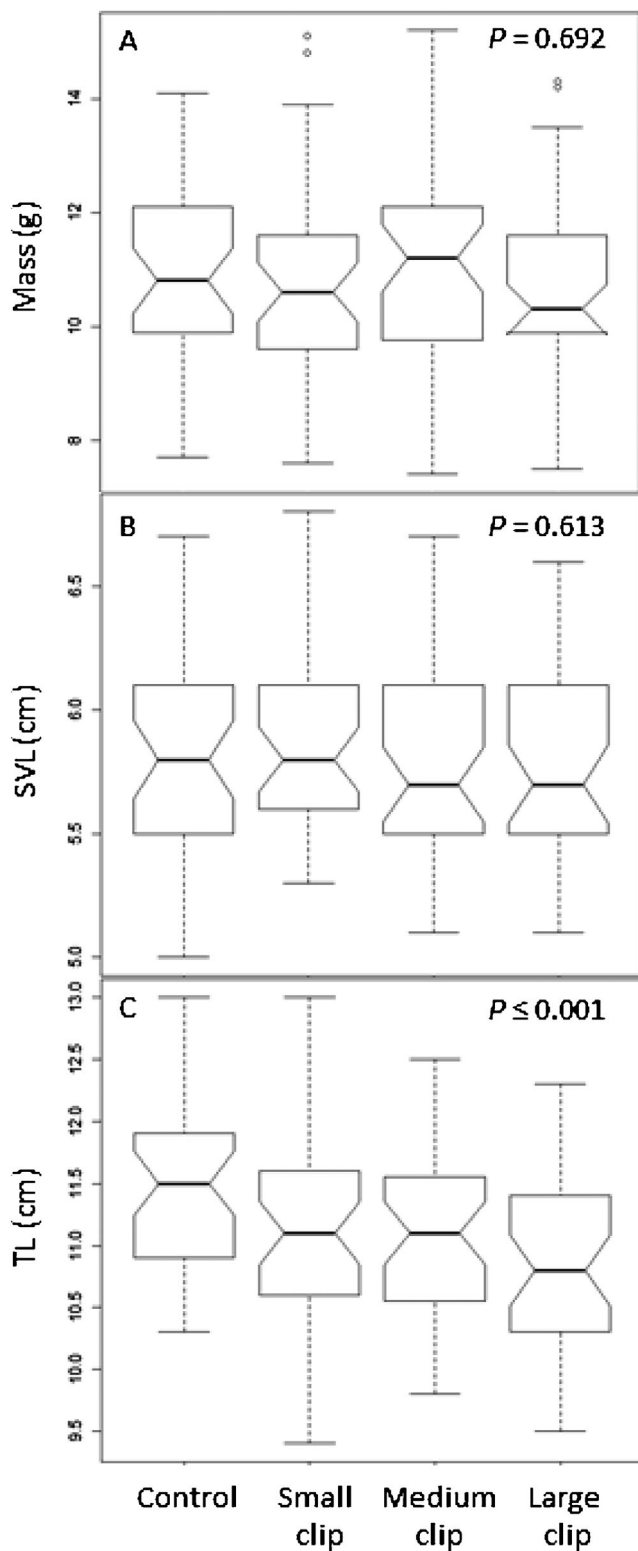
**Figure 1.** Experimental design of a semi-natural mesocosm experiment to evaluate the effects of tail-clipping on larval growth and survival in the California tiger salamander, 2012. All larvae came from 2 breeding crosses, 1 between colony adults and 1 between wild-caught adults (Jepson). We reared eggs from these crosses in the laboratory and randomly assigned hatchlings to the 4 tail-clipping treatments. The experiment commenced when we transferred larvae to cattle tanks, where they could compete for resources and potentially predate upon each other. Four larvae from each of the 4 tail-clipping treatments were housed together in each cattle tank, yielding 16 larvae per cattle tank. We stratified larvae by breeding cross type, but more eggs were available from the wild-caught cross than from the colony cross, and thus some cattle tanks had a 3:1 ratio of wild-caught:colony larvae rather than a 1:1 ratio. We set up 10 cattle tanks replicates.

group was compared to the clipping treatments in a  $2 \times 2$  contingency table ( $P = 0.919$ ).

The 1-way ANOVAs for differences in growth rate revealed that tail-clipping treatment had no significant effect on mass ( $P = 0.692$ ) or snout-vent length ( $P = 0.613$ ) but did have a significant effect on total length ( $P \leq 0.001$ ; Fig. 2). We found no initial, pre-clipping differences in average total length between the 4 experimental groups at the start of the experiment ( $P = 0.454$ ), so this difference in total length must have been a result of tail-clipping. Immediately following the tail-clipping, the individuals in the small, medium, and large clip treatments were an average of 7.5%, 11.1%, and 18.5% shorter than those in the control group, respectively. By the conclusion of the experiment, these same groups were 3.2%, 3.3%, and 6.1% shorter on average than the individuals from the control group. This indicates that across all 3 treatments the salamanders regenerated an average of 66% of the tail that was lost from clipping. Across all 4 treatments, the average salamander increased in total length by a factor of 2.52, indicating that tail-clipping did not inhibit active foraging and substantial growth.

## DISCUSSION

Our results detected no negative effects of tail-clipping on snout-vent length, mass, or survival. Thus, we conclude that tail-clipping is a reasonable approach for collecting genetic samples. We did find that the 10-mm clip decreased total length; however, snout-vent length is the primary metric of size in herpetofauna. These results are particularly compelling given that we took tail clips up to 10 mm in length (almost double the amount that current federal regulations allow to be taken from wild animals). In these cases, the clip constituted over 20% of the animal's body length, yet larval salamanders in semi-natural mesocosm settings were still able to more than double in length and regenerate most of their lost tail. Although other tissue sampling techniques can be used that do not affect locomotor performance, such as buccal swabs and blood puncture (Gallardo et al. 2012, Mendoza et al. 2012), these methods may be extremely difficult to perform on small larval amphibians. For California tiger salamanders and many related groups of salamanders (Ambystomatidae, Dicamptodontidae, and Salamandridae) where post-metamorphic animals are secretive but larvae are not, larval tail-clipping is an easy, effective



**Figure 2.** Effects of tail-clipping on larval growth of California tiger salamanders in a semi-natural mesocosm, 2012. We found no significant differences between tail-clipping treatments for either mass (A) or snout-vent length (SVL; B). Despite substantial regeneration, total length (TL) was shorter in the large clip treatment than in the control (C). Bold horizontal lines are medians; boxes show inner quartiles; notches mark 95% confidence intervals such that treatments with non-overlapping notches can be considered significantly different; whisker bars show 1.5 times the interquartile range (or maximum/minimum value if closer to the median); open circles are outliers.

source of genetic sampling, and we recommend it as the tissue sampling method of choice. Our results further suggest that larger tail clips, up to 20% of total length and yielding enough tissue for multiple analyses including most Next-Generation genomic approaches, may be acceptable even for endangered taxa. However, we advise caution in applying our methods to other species of salamander. Predation levels may be greater in the wild than they were in our mesocosms, especially when another larval salamander occurs in the community that has a size advantage due to a difference in phenology. For instance, larval marbled salamanders (*Ambystoma opacum*) feed upon spotted salamander (*A. maculatum*) larvae (Stenhouse et al. 1983); marbled salamanders are fall-breeders and hatch earlier than spotted salamander larvae. Still, being housed with other California tiger salamander larvae does provide some predation pressure since the larvae are known to be cannibalistic, even on larvae only slightly smaller than themselves (H. B. Shaffer and C. A. Searcy, unpublished data). We suggest that preliminary tail-clip trials should be performed on a sub-sample of study organisms before the actual experiment is conducted if feasible.

Although other forms of marking or genetic sampling, such as toe-clipping (Davis and Ovaska 2001, McCarthy and Parris 2004) have often proven to be detrimental, we found no negative effects of tail-clipping. At least 3 explanations seem plausible. First, salamanders are able to regenerate their tails both very quickly and very accurately. The response to tail injury in salamander larvae is rapid; within hours of tail loss, skin heals over the wound and the blastema (a region of rapidly dividing progenitor cells) is formed, and the regeneration machinery is fully operating by the end of the third week post-trauma (Zhang et al. 2000, Monaghan et al. 2007, Sehm et al. 2009). The rapidity of regeneration, combined with the unique urodele ability to fully regenerate the caudal nervous system, may explain the ability of salamanders to compensate for even relatively substantial tail loss. Second, larval salamander locomotion, which relies on waves of lateral undulation that move through the body and tail, is not strongly effected by losing a substantial portion of the tail. Although the tail tip experiences the greatest lateral displacement of any part of the body during locomotion (and therefore contributes to a disproportionately large degree to locomotor performance; Frolich and Biewener 1992), Marvin (2011) found in larval black-bellied salamanders (*Desmognathus quadramaculatus*) that tails 69% or more of their original length resulted in swimming speeds that were not significantly different from pre-tail loss performance. In the context of these results, California tiger salamanders in our 10-mm tail clip treatment lost about half of their tail, whereas salamanders in the 5-mm treatment lost about a quarter. Thus, the only salamanders that lost any swimming ability may have been those in our largest tail clip treatment, and given the rapid regeneration of the tail, this potential decrease was probably short-lived. Finally, our results may reflect the artificial nature of our mesocosm experiments. No natural predators occurred in our tanks besides conspecifics and ad libitum

food was available. Fitzpatrick et al. (2003) found that tail areas of larval barred tiger salamanders, a close relative of the California tiger salamander, are significantly correlated with acceleration, at least in small larvae. Given the importance of acceleration in avoiding insect predators that sometimes prey on salamander larvae, survivorship in our cattle tanks was possibly artificially inflated for tail-clipped larvae compared to natural conditions. However, acceleration is presumably also important in escape from conspecifics given the suction feeding mechanism that characterizes ambystomatid larvae (Shaffer and Lauder 1985), suggesting that even our most extreme manipulation level still allowed for effective escape from predators. Nevertheless, testing the escape behavior of California tiger salamander larvae in the presence of predators, such as larval insects, in addition to the presence of their conspecifics would be useful.

## MANAGEMENT IMPLICATIONS

Our results indicate that larval tail-clipping is an effective means of harvesting genetic samples from larval California tiger salamanders and likely many other larval salamander species. However, we recommend that researchers conduct additional studies before using tail-clipping on a broad scale with larvae of distantly related salamanders or other endangered species. Despite removing up to 20% of an individual's total length, we found no decrease in survivorship, and little or no decrease in mass or total length of larvae as they approached metamorphic size. This suggests that the current regulation limiting tail clips to 6.35 mm may be somewhat conservative. In considering what these limits should be, we feel that minimizing the effects of tail loss on individuals should be weighed against the benefits of having enough tissue to ensure that current and future molecular analyses can be conducted with a single sample. For example, given the reliability of molecular estimates of effective population size and population connectivity (Wang et al. 2009, 2011), studies that compare future and current samples could provide valuable insights into the effectiveness of ongoing species management practices. In addition, larger tail clips are particularly useful for Next-Generation population genomics work that scores tens of thousands of genetic markers rather than the handful that characterize most current studies. These types of studies are likely to increase in importance, particularly for amphibians that are rare or endangered and require active management. Unlike toe-clipping, larval tail-clipping appears to be a safe, effective means of acquiring adequate tissue for such studies with only marginal fitness costs, at least in this species.

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