Individual Recognition of Amphibians: Effects of Toe Clipping and Fluorescent Tagging on the Salamander Plethodon vehiculum

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Individual Recognition of Amphibians: Effects of Toe Clipping and Fluorescent Tagging on the Salamander *Plethodon vehiculum*

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ABSTRACT.—**Recognition of individual animals is essential for a wide variety of research and monitoring studies involving amphibians, but little information exists on the effects that marking methods have on survivorship, life history, and behavior. We evaluated toe clipping and subcutaneous injections of a fluorescent-elastomer for individual identification of the western red-backed salamander, *Plethodon vehiculum*, on Vancouver Island, British Columbia. In the laboratory, no confirmed mortality of marked or unmarked (control) salamanders occurred over 64 weeks. The number of toe clips lost as the result of regeneration increased steadily after 35 weeks postmarking, but few fluorescent marks were lost or misidentified. In the field, we recaptured more fluorescent-marked (60%) than toe-clipped (40%) salamanders from September 1997 to May 1998 but detected no differences in growth or spatial movements. In a second field experiment (27 April to 31 May 1999), toe-clipped salamanders gained less weight in relation to their initial body size than did fluorescent-marked and control salamanders. These data suggest that toe clipping affects the ability of individuals to take full advantage of optimal foraging conditions that prevail in May, which, in turn, might affect the quantity of stored energy reserves required for survival over adverse, dry periods in summer.**

Recognizing previously captured individuals is essential for estimating abundance from multiple samples (Krebs, 1989). Individual identification is also useful for obtaining information on growth rates, age at sexual maturity, frequency of reproduction, survivorship, and other life-history parameters and for investigating various aspects of behavioral ecology. For amphibians, a wide variety of identification and marking methods are available, including toe clipping, tattoing, branding, and tagging (Ferner, 1979; Donnelly et al., 1994). Because it is

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inexpensive and easy to do, toe clipping a unique combination of toes from each individual is commonly used in amphibian studies requiring individual recognition (Donnelly et al., 1994; Halliday, 1994). However, because toes can regenerate rapidly, the marks are not necessarily permanent (Ferner, 1979). Also, effects of toe clipping on survival, behavior, and recapture rates have received little attention, although some evidence suggests that morbidity or mortality may be significant (Clarke, 1972; Daugherty, 1976; Golay and Durrer, 1994; Reaser, 1995; Halliday, 1994, 1995). Of the alternative methods available for small amphibians, injectable polymer tagging methods are of particular interest because they are relatively noninvasive, and marks are long lasting (Woolley, 1973; Cecil and Just, 1978; Nishikawa and Service, 1988; Anholt et al., 1998).

We evaluated toe clipping and injectable fluorescent-elastomer marks with the western redbacked salamander, *Plethodon vehiculum*. These salamanders are small with the snout–vent length (SVL) of adults typically 40–50 mm and body mass < 2.5 g. Color patterns are superficially similar among individuals, which makes individual recognition through pattern mapping difficult. In the laboratory, we investigated mark-retention and survival. In two separate field experiments, we addressed mark retention, movement patterns, recapture rates, growth, and survivorship. In Experiment 1, we assessed the efficiency with which toe clipping and fluorescent marks could be applied and their retention through a 64-week period. Because initial results suggested that there might be differences in behavior between toe-clipped and fluorescent-marked salamanders, we carried out a second experiment (Experiment 2) to compare the effects of marking by these two methods with an unmarked (control) group.

Materials and Methods

Marking.—For toe clipping, we used surgical scissors to excise a unique combination of three toes from each salamander, removing no more than one toe per foot. After marking a salamander, we dipped the scissors in 95% ethanol to reduce the chance of spreading infections. No other antiseptics were used.

For fluorescent marking, we used subcutaneous injections of elastomer-dyes (from Northwest Marine Technology, Post Office Box 427, 976 Ben Nevis Road, Shaw Island, WA 98286) at six different body locations on the ventral surface: (1) immediately anterior to the right or left hind leg; (2) immediately posterior to the right or left hind leg; and (3) immediately posterior to the right or left foreleg. Together with three colors (red, orange, and yellow), these six positions, taken three at a time, result in 816 unique mark combinations. According to manufacturer's instructions, we mixed 10 parts elastomer to one part hardener, stirred for 1 min, and injected about 0.1 cc into the back of a 0.3-cc insulin syringe with a 29-gauge 12.7-mm needle. We injected the elastomer under the skin of the salamander using a specially designed holder, which greatly increased our ability to control the syringe (Fig. 1). After marking an individual salamander, we dipped the needle in 95% ethanol to reduce the chance of spreading infections. We applied and examined marks using a head-set magnifier (1.8-× magnification), and to enhance the visibility of fluorescent marks we used a long-wave UV-A lamp (Spectroline® battery-powered UV lamp, Spectronics Corporation, Westbury, NY; Model UV-4B) in a darkened box.

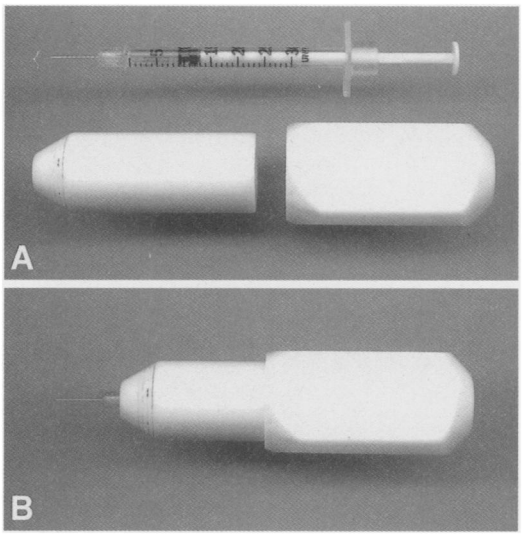

FIG. 1. Holder and 0.3-cc insulin syringe used for fluorescent marking (A). Syringe inside holder (B).

For field experiments, we mixed the fluorescent elastomers with hardener in the laboratory prior to use and kept the syringes in a cooler to prevent premature hardening. Between marking sessions, we kept the syringes containing unused elastomere frozen.

Laboratory Experiment.—On 12 June 1997, we collected 60 *P. vehiculum* (36–60 mm SVL, measured to the nearest 0.1 mm with calipers) from Chemainus Valley on southern Vancouver Island, British Columbia, Canada. We divided the salamanders randomly into three groups: (1) toe clipped; (2) fluorescent marked; and (3) control (unmarked).

We marked salamanders on 10 July 1997 and inspected marks for visibility each month for 64 weeks, after which the salamanders were re-

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leased at their original capture locations. At each monthly inspection, we weighed each salamander to the nearest 0.1 g either with a 10 g Pesola® spring scale (in 1997–1998) or with an Acculab® (Pocket Pro® 150-B) battery-powered electronic scale (in 1999).

We kept the salamanders individually in 1-liter glass Mason® jars loosely filled with moist moss and with an insect netting lid. Each week, we provided the salamanders with wingless fruitflies, small earthworms, or pinhead-sized crickets. All individuals received the same type of prey and similar numbers of prey items at each feeding. We changed the moss once each month. Fluorescent ceiling lights maintained a 12:12 L:D photoperiod in the laboratory. The air temperature varied from 6°C in winter to 24°C in summer (mean minimum temperature = 12.6°C; mean maximum temperature = 16.9°C).

Study Site for Field Experiments.—The study site was located in Goldstream Provincial Park (48°28′N, 123°32′W), about 20 km northwest of Victoria, Vancouver Island, British Columbia, Canada. The habitat consisted of mature forest dominated by western red-cedar (*Thuja plicata*) and big-leaf maple (*Acer macrophyllum*). From 28 September 1997 to 31 May 1999, we captured salamanders from 12 artificial cover objects that were installed in December 1991 in a previous study (Davis, 1996, 1997). Each cover object consisted of a 180-cm long baseboard of untreated Douglas fir (*Pseudotsuga menziesii*), covered with two top boards (Fellers and Drost, 1994; Davis, 1997). The spaces under the top boards on the baseboard and those under the baseboard on the soil were used by salamanders and could be inspected with minimal disturbance to the natural habitat. In the spring of 1999, we also searched five sections of Douglas fir logs (approximately 50 cm long, 30 cm in diameter), which had been placed at the site concurrently with the boards. All cover objects were within an area of 12 m × 19 m. We measured distances from the center of each cover object to the center of every other cover object and used these distances in the analysis of salamander movements.

Field Experiment 1.—We marked salamanders found during inspections of the cover objects on 28 September, 5 October, and 2 November 1997 and on 19 March, 9 April, and 26 May 1998. From a random start, we assigned individuals alternately to toe clipping or fluorescent marking. We chose a systematic rather than a random assignment to distribute salamanders caught from each individual cover object evenly between treatments. We did not use salamanders with deformities or injuries (apart from missing or regenerated tail tips).

We recorded the time required to mark each salamander, starting when holding the salamander and ready to begin marking and stopping when the mark had been applied and verified. Thus, marking time included positioning and restraining the salamander by one person, as well as handling the equipment. For fluorescent marking, it also included placing the syringe in the applicator and switching syringes when different color combinations were used.

We inspected the cover objects for recaptured salamanders on 26 September, 3 October, and 15 November 1998 and on 27–28 April, 4–5, 18, and 31 May 1999 (because of the large number of salamanders caught, the inspection was spread over two days on two occasions in 1999). For each salamander caught, we recorded the cover object identification number, sex (adults only), SVL (measured to nearest 0.1 mm with calipers), mass (as in the laboratory experiment), and reproductive condition (gravid or not for females). We also took notes of any injuries or distinguishing marks to aid in individual identification. In the fall of 1998 and the spring of 1999, we also photographed marked salamanders to compile an identification atlas and to confirm identities from dorsal patterns when necessary. We released each salamander at its original capture location within 15–45 min after capture.

Field Experiment 2.—Because initial analysis of data from Experiment 1 suggested that there might be differences in movements between toe-clipped and fluorescent-marked salamanders, we carried out a second experiment that included an unmarked, control group to establish a baseline of normal behavior. On 27–28 April and 4–5 May 1999, we assigned previously unmarked salamanders found under the cover objects into one of three identification methods: toe clipping; fluorescent marking; or pattern mapping (control). We assigned the first three salamanders randomly into one of the three methods each and then systematically repeated the pattern for subsequent salamanders. We included only salamanders with SVL ≥ 35 mm.

We marked salamanders in the toe-clipping and fluorescent-marking treatments as in Experiment 1. To identify individuals in the control group, we used slight differences in color patterns, especially dark flecking on the dorsal stripe. We photographed the salamanders and sketched any obvious distinguishing marks on a standardized drawing of a salamander outline. Also, we photographed each toe-clipped and fluorescent-marked salamander at first capture to identify individuals with ambiguous marks in subsequent captures.

To collect additional data on recaptured salamanders, we inspected the cover objects on 18 and 31 May 1999. We recorded the same infor-

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mation as in Experiment 1 for each salamander, except that all body mass measurements were taken with an electronic balance. To identify recaptured salamanders in the control group, we compared each unmarked salamander with photographs taken previously.

Data Analysis.—To compare the marking methods with respect to the number of correctly identified marks and the number of marks retained at the end of the laboratory experiment, we used χ^2 tests (Zar, 1996). We compared daily weight change of captive fluorescent-marked, toe-clipped, and unmarked control salamanders using ANOVA for repeated measures with identification method as the effect variable, initial SVL as the covariate, and 15 measures of the daily mass change as the response variables. We calculated daily mass change from previous masses of individuals taken on initial capture on 12 June 1997 and on 15 subsequent occasions 21–39 days apart from 4 August 1997 to 1 October 1998. Because the data violated the assumption of normality required by parametric tests, we carried out the test using both ranked and actual values of daily weight change and examined the results for congruence (Zar, 1996: 269–270).

To analyze the field data, we used the χ^2 test to compare frequency distributions of the number of captures per individual, moves between cover objects by individuals, and moves between wood and soil microhabitats by individuals using only one cover, between toe-clipped and fluorescent-marked salamanders in Experiment 1 and among toe-clipped, fluorescent-marked, and control salamanders in Experiment 2. We also used the χ^2 test in Experiment 1 to compare frequency distributions of residency time (period between first and last capture divided into 100-day intervals up to 700 days) for recaptured salamanders between the two marking methods.

We compared the number of days between the first and last capture, distance between two farthest captures (a rough index of home range size), and daily weight change of individuals between the two marking methods in Experiment 1 using the Wilcoxon signed-ranks test for unpaired samples (Zar, 1996); the data did not conform with the assumption of normality required by parametric methods. We used the Kruskal-Wallis test to compare residency time among the three identification methods in Experiment 2 (Zar, 1996). Daily weight change in Experiment 1, but not in Experiment 2 (see below), was uncorrelated with SVL, permitting simple comparison between marking methods. We used ANCOVA to compare growth rates (daily change in SVL) between the two marking methods for salamanders that were present for at least 100 days in Experiment 1. Marking method was the effect variable; initial SVL was the covariate; and either absolute daily growth rate or ranked daily growth rate was the response variable. We used the same method to compare the daily weight change among treatments in Experiment 2. Identification method was the effect variable; initial SVL was the covariate; and either actual daily change in weight or ranked daily change in weight was the response variable. We then examined the results for congruence (Zar, 1996). Neither growth rates nor rates of weight change were normally distributed, and we were unable to achieve normality by transformation.

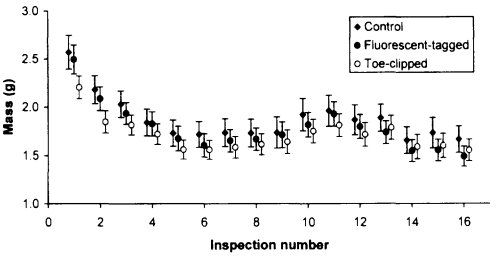

FIG. 2. Mass of unmarked control, fluorescent-marked, and toe-clipped salamanders in the laboratory during 16 inspections over a 476-day period from 12 June 1998 to 1 October 1998. Inspection 1 shows the pretreatment mass of salamanders, whereas inspections 2–16 show the posttreatment mass. Inspections were approximately 1 month apart (see text for exact dates).

For statistical analyses, we used Statsview 512® (vers. 1.0 for the MacIntosh) and Jmp In® (vers. 3.2.1., 1989–1997, Statistical Analysis Systems Institute, Inc.). In χ^2 tests, we applied the Yate's correction for continuity when df = 1 (Zar, 1996). In all tests, we set alpha to 0.05.

RESULTS

Laboratory Study.—There was no confirmed mortality of *P. vehiculum* during the experiment, but one salamander from the control group was missing on 8 September 1998 and either died or escaped. In addition, three salamanders from the fluorescent-marked group and one from the toe-clipped group apparently escaped through small holes in the netting lids, possibly made by crickets provided as food.

The daily weight change of salamanders in captivity varied significantly with their initial SVL, but the marking method had no detectable effect on this variable [Whole model (between subjects)—Wilks' lambda = 0.62, $F_{3,50} = 10.14$, $P < 0.0001$; Marking method—Wilks' lambda = 0.95, $F_{2,50} = 1.38$, $P > 0.2$; SVL—Wilks' lambda = 0.66, $F_{1,50} = 25.7$, $P < 0.0001$; Fig. 2]. The rate of weight change varied significantly over the

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TABLE 1. Mark retention of *Plethodon vehiculum* in captivity for 448 days (64 weeks). Animals with poor marks had marks that were ambiguous or difficult to decipher. Animals that escaped are not included.

Marking method	*N*	Number of salamanders: Decipherable mark	Lost or poor mark	Misidentified ≥ once
Fluorescent-marked	17	16	1	3
Toe-clipped	19	8	11	13

study period [Time (within subjects)—Wilks' lambda = 0.44, $F_{14,37} = 3.34$, $P < 0.002$]. However, the interaction between time and marking method was not significant (Wilks' lambda = 0.43, $F_{28,74} = 1.40$, $P > 0.1$).

On average, salamanders had gained weight on seven and lost weight on eight monthly inspections. The greatest weight loss occurred immediately after capture ($\bar{x} = -0.007$ g/day from 12 June to 4 August 1997, SD = 0.004 g, N = 60, all treatments combined). When they occurred, the weight gains from one monthly inspection to the next were modest (greatest mean weight gain = 0.005 g/day from 12 March to 10 April 1998, SD = 0.005, N = 59, all treatments combined).

More fluorescent-marked than toe-clipped salamanders retained clear marks 64 weeks after marking ($\chi^2 = 8.7$, df = 1, $P = 0.003$). Only one of the 17 fluorescent-marked salamanders present at the end of the experiment had lost a mark, whereas 11 of the 19 toe-clipped salamanders had lost or ambigious marks as a result of regeneration (Table 1). The one lost fluorescent mark disappeared during the first month after marking. One of the three marks of two other elastomer-tagged salamanders could not be seen on 1–2 previous occasions but was visible during the last inspection on 1 October 1998. Close inspection of these marks revealed that they were inadvertently injected deep into the musculature, thereby reducing their visibility. Near the end of the experiment, regeneration of toes made many of the toe-clip marks difficult to interpret, and we had to rely on slight differences in the shape of the regenerated toes for identification. The number of errors associated with deciphering toe clips was initially low but increased rapidly after 35 weeks postmarking. In contrast, the numbers of errors associated with deciphering fluorescent marks remained low throughout the study (Fig. 3).

Field Experiment 1.—In fall 1997 and spring 1998, we marked 115 salamanders by toe clipping and 115 salamanders with fluorescent-elastomers. Of these, 69 fluorescent-marked and 46

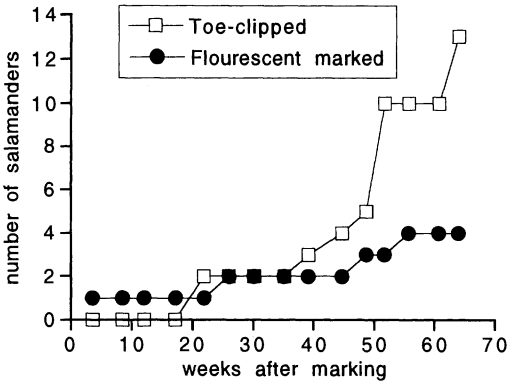

FIG. 3. Cumulative number of *Plethodon vehiculum* with ambiguous marks in the laboratory. Number of toe-clipped salamanders = 19. Number of fluorescent-marked salamanders = 17.

toe-clipped salamanders were recaptured 1–11 times during the study (until 31 May 1999). In total, there were 125 recaptures of toe-clipped and 212 recaptures of fluorescent-marked salamanders. Toe-clipped salamanders were recaptured less frequently than were fluorescent-marked salamanders (60% of toe-clipped vs. 40% of fluorescent-marked salamanders were never recaptured; $\chi^2 = 8.4$, df = 1, $P < 0.01$, individuals with > 2 captures combined). Also, fewer toe-clipped than fluorescent-marked salamanders were recaptured during seasons subsequent to marking ($\chi^2 = 6.6$, df = 1, $P < 0.025$, fall- and spring-marked salamanders combined; Table 2). The observed values denote minimum survival over winter for fall-marked salamanders and over summer for spring-marked salamanders; winter and summer are seasons when the salamanders are mostly inactive and are seldom found on the surface (Ovaska and Gregory, 1989).

The period from the first capture to the last recapture varied from 7–610 days and was similar for both marking methods (toe clipping: $\bar{x}$ = 298 days, SD = 193 days, N = 46; fluorescent

TABLE 2. Number of *Plethodon vehiculum* marked in the spring or fall that were recaptured during the subsequent activity season after summer or winter, respectively. Individuals recaptured only during the same season when marked are omitted.

	Marked in spring 1998		Marked in fall 1997		
	Fluorescent-marked	Toe-clipped	Fluorescent-marked	Toe-clipped	Total
Total no. marked	52	49	63	66	230
No. recaptured	29	18	16	8	71

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marking: $\bar{x}$ = 333 days, SD = 187 days, N = 69; $Z = -0.92$, $P > 0.3$, Wilcoxon signed-ranks test). When divided into 100-day intervals, the frequency distribution of the residency time was also similar for the two marking methods (χ^2 = 5.0, df = 6, $P > 0.5$).

Individual growth showed a strong, negative correlation with SVL at initial capture (salamanders marked in fall 1997: $r^2 = 0.58$, $F_{1,43} = 58.3$, $P < 0.001$; salamanders marked in spring 1998: $r^2 = 0.31$, $F_{1,45} = 20.4$, $P < 0.001$). Small salamanders grew rapidly, whereas most adult-sized salamanders (SVL > 40 mm at first capture) grew more slowly or showed no growth. Marking method had no detectable effect on daily growth rate of salamanders [salamanders marked in fall 1997: ANCOVA with SVL as covariate and actual growth rate as response variable: $F_{2,42}$ (Whole model) = 29.2, $P < 0.0001$; $F_{1,43}$ (SVL) = 52.1, $P < 0.0001$; $F_{1,43}$ (Marking method) = 0.59, $P > 0.4$; salamanders marked in spring 1998: $F_{2,44}$ (Whole model) = 11.2, $P < 0.0001$; $F_{1,45}$ (SVL) = 22.1, $P < 0.0001$; $F_{1,45}$ (Marking method) = 1.7, $P > 0.2$]. We obtained similar results using ranks of the growth rate as the response variable [salamanders marked in fall 1997: $F_{1,43}$ (SVL) = 39.60, $P < 0.0001$; $F_{1,43}$ (Marking method) = 0.9, $P > 0.3$; salamanders marked in spring 1998: $F_{1,45}$ (SVL) = 20.0, $P < 0.0001$; $F_{1,45}$ (Marking method) = 2.8, $P = 0.1$].

In the spring of 1998, daily weight change of salamanders was uncorrelated with SVL at first capture ($r^2 = 0.006$, $F_{1,30} = 0.2$, $P > 0.6$). The mean daily weight change of salamanders marked on 19 March or 9 April 1998 and recaptured on 26 May 1998 was somewhat lower for toe-clipped than for fluorescent-marked individuals, but there was no statistical difference between the two groups ($Z = -1.6$, $P > 0.1$, Wilcoxon signed-ranks test). Insufficient numbers of recaptures during the fall of 1997 prohibited a similar comparison.

Most salamanders were highly site specific and used only one cover object throughout the study. Six of 25 fluorescent-marked and one of 21 toe-clipped salamanders had changed cover objects when first recaptured during the season when marked (spring or fall; Table 3A). Over the entire study (until 31 May 1999), 18 fluorescent-marked salamanders changed cover objects once and six did so twice, whereas nine toe-clipped salamanders changed cover objects once and one did so twice. The difference between the two marking methods was not statistically significant (Table 3A–B).

The distance between two farthest captures for individual salamanders ranged from zero to 10.3 m and was uncorrelated with the number of captures/individual ($r^2 = 0.0006$, $F_{1,113} = 0.07$, $P > 0.7$); its relationship to the residency time of individuals was also poor ($r^2 = 0.01$, $F_{1,113} = 1.2$, $P > 0.2$). The distance between two farthest captures was similar for toe-clipped and fluorescent-marked salamanders ($Z = -1.6$, $P > 0.1$, Wilcoxon signed-ranks test).

TABLE 3. Number of salamanders in Experiment 1 that had changed cover objects by the first recapture during the same season (autumn or spring) when marked (A), and that changed cover objects during the study from September 1997 to May 1999 (B).

(A) Number of individuals that had moved by first recapture during the season when marked.

No. of moves	Fluorescent-marked	Toe-clipped	Total
0	25	21	46
1	6	1	7
Total	31	22	53

$\chi^2 = 1.3$, df = 1, $P > 0.95$

(B) Total number of moves between cover objects by individual salamanders.

No. of moves	Fluorescent-marked	Toe-clipped	Total
0	45	36	81
1–2	24	10	34
Total	69	46	115

$\chi^2 = 1.7$, df = 1, $P > 0.2$

On recapture, five of 69 fluorescent-marked salamanders had lost one of their three marks, and 13 had ambiguous marks. Ambiguous marks resulted either from subcutaneous movement of all or part of a mark from its original position (N = 9) or from our inability to locate one of the three marks, although visible on at least one subsequent recapture (N = 4). Because of regeneration, three of 49 recaptured, toe-clipped salamanders had lost a mark, and seven had marks that were difficult to decipher. We were able to determine the identity of these salamanders based on a combination of the remaining marks, body size, and natural dorsal markings in reference photographs. We first observed lost or ambiguous toe clips 161 days after marking and lost or ambiguous fluorescent marks 21 days after marking. Four of five lost fluorescent marks were detected on the first recapture. Besides salamanders with lost or ambiguous marks, we were unable to match seven fluorescent-marked and eight toe-clipped recaptures with previously marked salamanders.

Toe clipping took an average of 38 sec per salamander (SD = 11 sec; range: 19–75 sec, N = 114), whereas fluorescent marking took an average of 137 sec (SD = 32 sec; range: 75–210 sec, N = 114), including equipment assembly by one person.

Field Experiment 2.—The toe-clipped, fluores-

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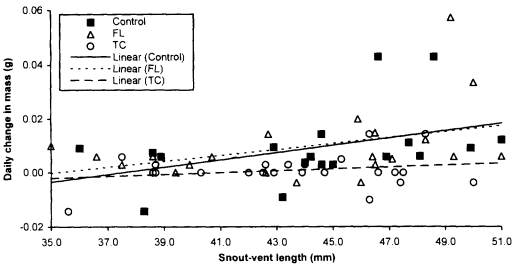

FIG. 4. Daily change in mass of unmarked control salamanders and salamanders marked with either fluorescent-marks (FL) or toe clipping (TC) in the field from 27 April to 31 May 1999. N = 18 (Control), 21 (FL), 23 (TC).

cent-marked, and control treatments each had 32 salamanders; there were no gravid females in any of the groups. From 4 May to 31 May 1999, we recaptured 39 toe-clipped, 45 fluorescent-marked, and 40 control salamanders 1–4 times. The frequency distribution of individuals caught only once versus those caught repeatedly was similar for all identification methods (χ^2 = 1.7, df = 2, $P > 0.2$; individuals caught 2–4 times combined). Residence time, measured from the first to last capture, was also similar among treatments (χ^2 approximation = 0.4, df = 2, $P > 0.8$, Kruskal-Wallis test).

Daily weight change during 27 April to 31 May 1999 showed a weak, positive correlation with the initial SVL, and larger salamanders gained more weight than did smaller salamanders (r^2 = 0.13, $F_{1,60}$ = 8.7, $P < 0.025$; Fig. 4). Daily weight gain was significantly lower for toe-clipped salamanders compared to fluorescent-marked and control salamanders [ANCOVA with SVL as covariate and weight change/day as the response variable: $F_{3,58}$ (Whole model) = 5.8, $P < 0.002$; $F_{1,60}$ (SVL) = 8.2, $P < 0.006$; $F_{2,59}$ (Marking method) = 3.9, P = 0.025]. We obtained similar results using ranks of the daily weight change as the response variable [$F_{3,58}$ (Whole model) = 7.9, P = 0.0002; $F_{1,60}$ (SVL) = 4.4, P = 0.04; $F_{2,59}$ (Marking method) = 8.9, P = 0.0004].

Most recaptured salamanders used only one cover object during the experiment. At first recapture, only four salamanders had changed cover objects (Table 4A). A fluorescent-marked juvenile and an adult male had moved 1.8 m and 1.5 m, respectively; a toe-clipped juvenile had moved 1.8 m; and an adult female from the control group had moved 2.7 m. From 27 April to 31 May 1999, the number of moves made by individual salamanders among cover objects was uncorrelated with the number of captures (r^2 = 0.016, $F_{1,60}$ = 1.0, $P > 0.3$). Movement patterns of salamanders were similar regardless of identification method (Table 4B). Movement distances by the seven salamanders that changed cover objects ranged from 1.5 m in the fluorescent-marked treatment to 2.7 m in the control treatment; no salamander changed cover objects more than once.

TABLE 4. Number of *Plethodon vehiculum* in Experiment 2 that had changed cover objects by first recapture (A) and that changed cover objects during the study from 27 April to 31 May 1999 (B). Unmarked control salamanders were identified through pattern mapping from photographs.

(A) Number of individuals that had moved by first recapture during the season when marked.

No. of moves	Fluorescent-marked	Toe-clipped	Control	Total
0	19	22	17	58
1	2	1	1	4
Total	21	23	18	62

(B) Total number of moves between cover objects by individual salamanders.

No. of moves	Fluorescent-marked	Toe-clipped	Control	Total
0	18	22	16	56
1	3	1	2	6
Total	21	23	19	62

Two fluorescent-marked salamanders had each lost a mark, and two others had ambiguous marks resulting from subcutaneous mark movements. One toe-clipped salamander lost an additional toe from unknown causes, resulting in an ambiguous mark. We confirmed the identity of all the above individuals from photographs.

DISCUSSION

The majority of both fluorescent-marked and toe-clipped *P. vehiculum* retained their marks for the duration of the study (up to 610 days in the field and 448 days in the laboratory). However, some loss of marks or reduction in their visibility occurred with both methods. Anholt et al. (1998) reported that 15% of the fluorescent-elastomer marks in anuran larvae were lost within the first eight days. In our experiments, the loss of fluorescent marks also occurred shortly after marking, possibly as a result of improper application. However, we did not observe fewer marks lost with increasing experience of the marker; two of 21 recaptured individuals in field experiment 2 had lost marks, although we had gained considerable experience with the method (> 200 animals marked). Loss of toe clips resulted from regeneration, which was first noticeable 153 days after marking in the laboratory and 161 days after marking in the field.

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This loss can be expected to continue until most marks are obscured.

In the laboratory, neither fluorescent marking nor toe clipping resulted in mortality of *P. vehiculum*. We noticed no obvious infections or necrosis following marking by either method, such as reported for the natterjack toad (*Bufo calamita*) after toe clipping (Golay and Durrer, 1994). However, toe stumps of some *P. vehiculum* were swollen to about twice their normal width and appeared inflamed, as also reported for toe-clipped *Rana luteiventris* (formerly *pretiosa*; Reaser and Dexter, 1996). In the field, some toe-clipped salamanders had swollen toe stumps for up to 240 days after marking, but subsequent recaptures of many of these individuals indicated that the toes eventually healed and regenerated in an apparently normal manner.

In May 1999, individual toe-clipped *P. vehiculum* gained less weight in relation to their initial body size than did fluorescent-marked and control salamanders. Based on the number of captures, the surface activity of *P. vehiculum* peaks in May, and most salamanders disappear from the surface during the dry summer months (July and August; Ovaska and Gregory, 1989; Davis, 1996). The accumulation of sufficient energy reserves prior to estivation might be essential for survival, especially in years with prolonged summer drought. Our data suggest that toe clipping affects the ability of individuals to take full advantage of optimal foraging conditions that prevail in May and hence might affect the quantity of stored energy reserves. Toe clipping may similarly result in decreased weight gain in the fall when salamanders accumulate energy reserves for overwintering, but we lacked sufficient recaptures after marking in the fall of 1997 to examine this possibility.

In the field, we recaptured significantly fewer toe-clipped than fluorescent-marked salamanders, both within a season and in subsequent seasons. This pattern suggests higher mortality of toe-clipped than fluorescent-marked salamanders. Alternatively, obliteration of toe-clip marks by regeneration might have been responsible for this pattern. However, we believe that this explanation is unlikely because regenerated toes were usually slightly different in appearance from normal toes and because, in the laboratory, all salamanders could readily be recognized as recaptures from their multiple toe clips 64 weeks after marking. Emigration of toe-clipped salamanders out of the study area is also an unlikely explanation for this pattern.

Movement patterns of toe-clipped and fluorescent-marked *P. vehiculum* were similar over the 20-month field study from September 1997 to May 1999. In a second, month-long experiment in the spring of 1999, movements of unmarked (control) salamanders were similar to those of marked individuals. Throughout the study, most individuals showed high fidelity to particular artificial cover objects and were often recaptured at the same location. Site-fidelity and short average movements (< 2 m) over periods of years have been reported for individual *P. vehiculum* in the natural habitat in Goldstream Provincial Park (Ovaska and Gregory, 1989) and other sites on Vancouver Island (Davis, 1996, 1998). These data suggest that the use of artificial cover objects, rather than natural cover, did not bias our movement data.

Although most studies have found little or no evidence for significant adverse effects on survivorship and behavior in amphibians following toe clipping (Castellano and Giacoma, 1993; Lemckert, 1996; Reaser and Dexter, 1996; van Gelder and Strijbosch, 1996; Schlaepfer, 1998; Lûddecke and Amézquita, 1999), there is some evidence that adverse effects may occur (Halliday, 1995). For example, Clarke (1972) found an inverse correlation between the number of toes excised and the recapture rate of Fowler's toad (*Bufo woodhousei fowleri*), suggesting that toe clipping either affected behavior or reduced survivorship. However, Clarke (1972) did not randomize his marking procedure, and other interpretations could explain his results (Reaser, 1995; van Gelder and Strijbosch, 1996). J. C. Underhill (pers. comm. cited by Daugherty, 1976) found weight loss in leopard frogs (*Rana pipiens*) following toe clipping, but no details have been published. Golay and Durrer (1994) reported that 18% of toe-clipped natterjack toads (*Bufo calamita*) suffered infection and necrosis, sometimes involving the entire limb. These observations and the results of our field study suggest that problems with toe clipping may be more widespread than previously believed because of untested assumptions about behavior and survivorship.

Our study shows that toe clipping can result in subtle effects that might reduce survival in the field. Therefore, we do not recommend its use routinely in mark-recapture studies of plethodontid salamanders. Fluorescent marking, either alone or in combination with pattern mapping, might be a suitable alternative. The effects of toe clipping and other marking methods on the survival and behavior of other amphibians are generally unknown, but marking methods should be carefully assessed before being used in mark-recapture studies.

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