ment with vegetation also has been observed with the satin-ribbon band (M. P. Hayes and K. R. McAllister, pers. comm). Differences among studies may reflect the risks posed by different types of vegetation. All but one of my study sites had limited vegetation besides duckweed.

Both techniques described here have limitations, but the advantage of the arm-band attachment clearly lies with gravid females for which waist-band options probably interfere with oviposition. Since abrasion with the arm-band technique can occur rapidly, I recommend application of this technique for intervals no longer than a week. As such, the arm-band technique is probably best applied to females of explosive breeding anurans, which generally oviposit with a high degree of synchrony over a few days. Waist-band attachments appear less likely to induce abrasions and thus remain the preferred class of attachment techniques for non-gravid females. However, abrasion risk with the waist-band attachment is significant over long time intervals, and application of this technique should be done only where relatively frequent inspection (weekly) of frogs is possible.

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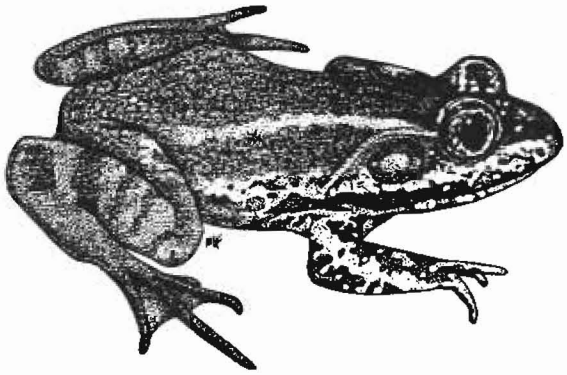

Rana virgatipes (Carpenter Frog). Illustration by Michael G. Frick.

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Use of Oxytetracycline in Batch-Marking Post-Metamorphic Boreal Toads

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Captive rearing and translocation have been discussed as management tools in the recovery of the natterjack toad (*B. calamita*), common frog (*Rana temporaria*), common toad (*B. bufo*), and boreal toad (*B. boreas*)(Cooke and Oldham 1995; Denton et al. 1997; and Loeffler 1998, respectively). Translocation is fraught with many concerns and difficulties (Dodd and Seigel 1991), foremost among these is determining the survival rate of released individuals. To estimate survival, long term monitoring is necessary (Dodd and Seigel 1991) as well as the availability of a reliable method with which to mark individuals before translocation or release (Burnham et al. 1987). Marking allows the translocated individuals to be distinguished from a resident population or potential emigrants, and allows recaptures to be assigned to a particular experimental treatment or cohort release.

Current methods for marking amphibians require the handling of each individual. Toe clipping (Donnelly et al. 1994; van Gelder and Strijbosch 1996) and PIT tags (Brown 1997; Corn et al. 1997) have been used to effectively mark adult anurans, but these techniques are ill-suited for new metamorphs. Metamorphs are too small to receive implanted PIT tags, and toe clipping is labor intensive, difficult to perform and can be injurious to small animals. Additionally, young anurans may partially regenerate clipped toes (e.g., *Bufo boreas*, C. Carey and K. Scherff-Norris, pers. comm.), causing identification errors. Fluorescent dye has been used to mark small amphibians with limited success (Schlaepfer 1998). Recently, fluorescent dye has been mixed with semi-liquid elastomer and then injected under the skin of several amphibians, successfully marking them (Anholt et al. 1998; Woolley 1973; Davis and Ovaska 1998). However, amphibian reintroduction may involve thousands of individuals, reducing the feasibility of a mark which must be delivered individually. A batch marking method is needed that allows released individuals to be identified at least until they attain a size where, upon recapture, adult marking methods can be applied.

Tetracycline has been used to batch mark hatchery-reared fish by immersion bath, through diet or by injection (Peterson and Carline 1996; Unkenholz et al. 1997; Weber and Ridgeway 1967). Tetracycline deposition in bone matrix follows the pattern of new mineralization (Frost 1969; Recker 1983) providing a label which can be seen with fluorescent microscopy (Frost 1969; Minaire et al. 1974; Recker 1983). Weber and Ridgeway (1967) determined that low doses of tetracycline caused little distress or damage to

ish. Unkenholz (1997) reported <1% mortality in perch fingerings immersed in oxytetracycline solutions of 309–748 mg/L. The first studies to use tetracycline labeling in amphibians were by Smirina (1972) and Francillon (1979, 1980) on *Rana temporaria* and *Triturus cristatus cristatus*, respectively. The use of tetracycline in amphibians and reptiles was reviewed by Frazier (1985a).

The effectiveness of tetracycline batch marking in fish and the high permeability of amphibian skin led us to evaluate the efficacy of oxytetracycline for batch marking young boreal toads. Oxytetracycline was chosen because of its recent, successful use in marking experiments (e.g., Unkenholz et al. 1997). Additionally, oxytetracycline has a low systemic toxicity (Suzuki and Mathews 1966; Weber and Ridgeway 1967), no "local effect on calcification" (Suzuki and Mathews 1966), and has a high intensity of fluorescence (Saxen 1969). Tetracycline markers have been used in rabies vaccinations and are acceptable in the food animal industry (L. Wolfe, DVM, pers. comm.). While the use of tetracycline ultimately requires a toe clip to read the mark, adult animals often are toe clipped as part of mark–recapture population studies (Donnelly et al. 1994). Handling stress and toe regeneration are presumably reduced in the larger adults and the excised toes would be available to determine the presence or absence of a batch mark.

Boreal toadlets were reared in captivity at the Research Fish Hatchery in Bellvue, Colorado by the Colorado Division of Wildlife from eggs collected in Clear Creek County, Colorado, USA (UTM: Zone 13, 429324 E, 4402598 N). Tadpoles and toadlets were maintained in outside raceways. Sixty, approximately three-month old toadlets were selected randomly from the raceway. Toadlets were weighed to the nearest 0.01 g and assigned randomly to one of six treatments (Table 1).

Oxytetracycline HCL (OXY-TET® 100, Anchor Division Boehringer Ingelheim Animal Health Inc., St. Joseph, Missouri, USA) baths (5–15 mm deep) of three concentrations were prepared using filtered water in clean plastic containers with ventilated lids. Toadlets were placed in the baths and left undisturbed in an environmental chamber (6 or 24 h).

After the baths, toadlets were rinsed with filtered water and placed in corresponding aquaria. Clean aquaria (45.5 L) were furnished with a layer of aquarium gravel (rinsed twice with filtered water), a layer of plastic lighting grating (approximately 10 mm x 10 mm mesh, washed with mild detergent and chlorine bleach, rinsed in filtered water and dried), and a layer of peat moss. Filtered water was added to the level of the grating. A moisture gradient was provided by slightly elevating the substrate at one end. Toadlets were fed 5 d per wk on live, first-instar crickets dusted with Septavite® vitamin powder. Water was changed once per month for the first month and once per wk thereafter. Moss was changed once per month and aquaria were misted occasionally with filtered water. Aquaria were kept in an environmental chamber for the entire 24 wk experiment (12 h:12 h light:dark cycle; 22°C during light and 15°C during dark; humidity: 96.6 ± 0.1%). The position of the aquaria in the environmental chamber was randomized and rotated each week.

After 24 weeks, the 3rd toe on the left front foot and the 2nd toe on the right hind foot were removed (Donnelly et al. 1994) from each toadlet, placed in ethyl alcohol, and kept in opaque containers. If toadlets died before the end of the experiment, toes were collected and analyzed. Only data from front toes were used in the analyses, except where data were missing due to difficulties in histological preparation (N = 6). In these cases, data from a hind toe were substituted.

Toes were processed using a methyl methacrylate method for calcified bone (Aquino, unpubl.; Ruhl-Fehlert and Ludl 1987; Sanderson et al. 1990). Methacrylate blocks were cut to approximately 1 x 1 cm squares. The side containing the bone was ground by sanding belt and by hand (wet grinding paper P800 and P1000) until a clear cross section of bone was visible on a 20x microscope. Each sample was numbered and placed in a small, opaque envelope.

Samples were examined under a Leitz microscope (36x) with a 100 W mercury source (Short Arc Lamp Power Supply, E. Leitz Co. Rockleigh, New Jersey, USA) using 400–440 (blue violet) excitor filter with a 470 barrier filter. Samples were scored and assigned numeric values as follows: 5) very good label; 4) good label; 3) poor label; 2) ambiguous fluorescence, unknown if label or autofluorescence; 1) no fluorescence or autofluorescence, bluish and diffuse; or 0) missing samples. Scoring was based on brightness and color. All samples were randomized and then examined "blind"; the scorer (EM) did not know which block or treatment she was examining. Data were analyzed using ordinal logistic regression (Agresti 1996) under a proportional odds model (SAS 1989). A design matrix was supplied to account for the categorical nature of the treatments (see, for example SAS 1989:1099–1101). Significance levels were set at 0.05 for single comparisons and 0.006 (corrected using the Bonferroni method) for multiple comparisons.

Initial masses of toadlets in the 6 treatments were not different (Table 1; $F_{5,54} = 0.270$, $P = 0.927$). Growth during the experiment was uniform among treatments; the final masses averaged at least three times the starting masses and were not different among treatments ($F_{4,43} = 0.573$, $P = 0.683$).

All the toadlets in treatment 2 died in February, probably due to a buildup of ammonia in the tank. We tested the water in all the tanks for ammonia (Jr. Ammonia Water Test Kit®, Wardley Corporation, Secauscus, New Jersey, USA). The test indicated ammonia levels in excess of 5.0 mg/L in the treatment 2 aquarium.

TABLE 1. List of treatments and mean mass of toadlets present at the start and end of experiment. * Approximate mass at death after 11 wks of treatment.

Treatment	Mean mass of toadlets (S.E., N) Start	End
1) Control A: 6 h filtered water	0.69 (0.07, 10)	2.61 (0.22)
2) 6 h oxytetracycline (1000 mg/L)	0.80 (0.07, 10)	0.77 (0.08)*
3) Control B: 24 h filtered water	0.71 (0.12, 10)	2.37 (0.23)
4) 24 h oxytetracycline (250 mg/L)	0.72 (0.05, 10)	2.23 (0.16)
5) 24 h oxytetracycline (500 mg/L)	0.75 (0.08, 10)	2.37 (0.13)
6) 24 h oxytetracycline (1000 mg/L)	0.74 (0.07, 10)	2.32 (0.18)

The other aquaria had ammonia levels (≤ 0.25 mg/ L). Toadlets were frozen at death and later thawed, blotted dry, and weighed. One toadlet in controls 1 and 3 also died in February. These were the smallest animals and may have starved.

Bone sections of toadlets exposed to tetracycline usually (36/39 = 92%) had distinguishable fluorescent labels (Figs. 1 and 2). Neither tetracycline concentration nor exposure time influenced the detectability or brightness of the label ($P \geq 0.038$). Control A (6 h) differed significantly from treatment 2 (6 h; $\chi_1^2 = 5.60$, $P = 0.018$), control B (24 h) differed significantly from treatments 4, 5, and 6 ($P \leq 0.002$), and control A (6 h) did not differ from control B (24 h; $\chi_1^2 = 2.57$, $P = 0.108$; Table 2).

Autofluorescence was a significant problem. In 3 of 39 treated samples, we were unable to determine whether the fluorescence resulted from the label or autofluorescence. Eleven of 20 control samples showed no fluorescence or only a diffuse, bluish glow indicating autofluorescence but in 9 of 20 control samples we observed fluorescence indistinguishable by eye from samples with labels. Three of the 9 fluorescing control samples and 3 of the clearly labeled samples were chosen at random, sectioned (10 μm), mounted, examined microscopically, and photographed. In sections of two control samples, the fluorescence appeared diffuse and bluish suggesting that the tissue was autofluorescing, but the third control section appeared to have a label. Color slides of these samples and the labeled samples revealed a difference between control bone and labeled bone, with all labeled specimens being distinctly more gold in color than the autofluorescence in the controls. One roll of film was used for all slides; the same exposure and ASA was used on all slides.

We found that oxytetracycline labeled the bones of soaked toadlets, but under low power magnification of the blocks, it was sometimes difficult to distinguish the yellow gold color of the oxytetracycline label from the natural, bluish-green, diffuse autofluorescence of the bone. These results, which agree with an experiment conducted concurrently on pickerel frogs (*Rana palustris*) and green frogs (*R. clamitans*) by Hatfield et al. (unpubl.), indicate that this method is not completely reliable.

Yellow green autofluorescence has been detected in turtles, but it was less intense and more diffuse than the label fluorescence at the line of active bone growth, where the label is expected (Frazier 1985b). In our study, neither oxytetracycline concentration nor length of treatment influenced the brightness of the label, contrary to Frazier's (1985a) findings. This may have been partly due to method. Frazier injected tetracycline whereas we soaked animals in a oxytetracycline solution. We soaked the toadlets in specific concentrations of oxytetracycline because toads absorb water readily through their skin and other methods of administration such as intramuscular, subcutaneous injection or oral dosing are problematic due to the size of the toadlet and the time necessary to handle thousands of animals. Although Milch et al. (1958) found no evidence that the route of administration affected the distribution of the label, the experimental toadlets were occasionally observed to place a limb on the side of the container, out of the solution. This may have affected the standardized uptake of the oxytetracycline and may have been related to the concentration of the solution. Hatfield et al. (unpubl.) found the soaking method to be ineffective. According to Milch et al. (1958) variation in fluorescence occurs between species and age in tetracycline labels.

FIG. 1. Cross section of phalange of boreal toadlet (24 h exposure in 1000mg/L tetracycline bath) viewed at 20x magnification under fluorescent light. Tetracycline label is visible as bright line in the cortex, below the periosteal layer, marrow tissue visible in center.

TABLE 2. Number of samples in each treatment for each label category: assigned ordinal values in brackets.

Treatment	N	very good label [5]	good label [4]	poor label [3]	auto- or label [2]	none or auto-fluorescence [1]	missing samples [0]
1) Control A: 6 h filtered water	10	0	0	0	6	4	0
2) 6 h oxytetracycline (1000 mg/L)	10	4	4	1	1	0	0
3) Control B: 24 h filtered water	10	0	0	0	3	7	0
4) 24 h oxytetracycline (250 mg/L)	10	6	2	1	0	0	1
5) 24 h oxytetracycline (500 mg/L)	10	0	7	2	1	0	0
6) 24 h oxytetracycline (1000 mg/L)	10	3	5	1	1	0	0

FIG. 2. Cross section of phalange of boreal toadlet (control, 24 h exposure in filtered water) viewed at 20x magnification. No tetracycline label visible.

The toadlets in this experiment were within 1 wk of the same age. There may have been an aquarium effect as all toadlets in each treatment were housed in the same aquarium. However, we believe this possibility is minimized given our care in cleaning the aquaria before the experiment and our randomization of aquaria position during the experiment.

This method is impractical as a field tool for several reasons. First, the toe must be processed and viewed in the laboratory. This introduces complexity and considerable cost, especially because thin sections and photography may be necessary to distinguish between a labeled bone and an autofluorescing bone. Histological costs were US $850.00 (120 specimens) plus an additional cost of US $7.00 per slide for the thin sectioning. Microscope time and photography of the sections were donated by CSU. Treatment costs including aquaria set up, oxytetracycline, and food was approximately US $450.00.

Second and perhaps most importantly, marks may appear ambiguous; unmarked toads can easily be scored incorrectly as having been marked and marked toads may be scored as unmarked. This violates an important assumption in all capture-recapture models (Pollock et al. 1990). The overall mis-identification rate for microscopic (36x) examination was 20% (Table 2). Due to cost constraints, we applied additional identification methods (e.g., thin sectioning and photography) to a small subsample; three of the potentially mis-identified specimens and three of the labeled and correctly identified specimens. These methods allowed us to differentiate between label and autofluorescence and determine that the three mis-identified specimens were exhibiting autofluorescence. Unfortunately, these results cannot be extended beyond our study due to sample size.

Finally, the longevity of tetracycline labeling has not been tested in amphibians although tetracycline labeling in green turtles (*Chelonia mydas*) was evident one and three years after treatment for live and frozen specimens respectively (Frazier 1985b). Although we do not recommend this particular method of tetracycline marking, batch marking with an appropriate method (e.g., Taylor and Deegan 1982) is a sound technique to use with very small or juvenile anurans.

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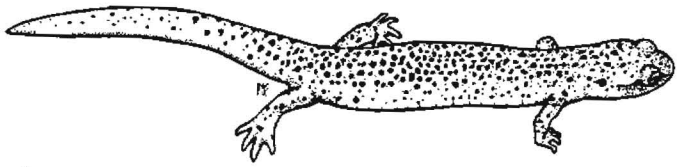

Pseudotriton ruber (Red Salamander). Illustration by Michael G. Frick.

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Modifications to the Stomach Flushing Technique for Turtles

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Determining fractional composition of dietary items is necessary when developing a complete ecological inventory for a species. Techniques for gathering data on food habits of turtles include direct observation, examination of fecal material (Demutl and Buhlmann 1997), and collection of stomach contents (Legle 1977; Parmenter and Avery 1990). Examination of the digestiv tract contents has advantages over other methods (Parmenter an Avery 1990)

As with other species (Demuth and Buhlmann 1997; Killebre 1991), we experienced problems with the stomach flushing metho in our dietary studies of the Texas river cooter (*Pseudemys texan* and the red-eared slider (*Trachemys scripta elegans*). Problem encountered ranged from little or no contents obtained to seriou internal damage to the turtles. Here we offer modifications to th stomach flushing technique which enhance its effectiveness.

Initially, we used a modification of Legler's technique and restraining device similar to that described by Parmenter (1980 The restraining table (60 cm x 28 cm) was tilted at a 35 degre angle allowing gravity to facilitate the flow of contents from th stomach. A turtle was secured to the table with a web strap and non-skid pad mounted on the table surface prevented the turt from sliding out of the webbing. A hand operated fluid transfe pump, mounted on the side of a laboratory bench, supplied wat for flushing through a small animal feeding tube (3.5 mm OI (Fig. 1).

Difficulties were encountered when using a hook and cable a paratus (Parmenter 1980) to extend the neck and hold open t mouth of an agitated turtle. These included trauma to the jaws a palate. Additionally, rupture of the esophagus due to blockage the flushing tube and food mass was noted in two post-procedu necropsies.

Administration of a tranquilizer, ketamine HCl, significan reduced this problem. Ketamine HCl is a dissociative anesthe (Bennett 1996) which is currently a federal schedule III drug quiring a prescription from a licensed veterinarian. Individu were injected intramuscularly in the left forelimb with 25mg ketamine HCl (Aiello 1998; Bennett 1996). The effect of the dr evinced by total relaxation with head extended, was noted in 2 30 minutes. The sedated turtle was positioned (carapace do head oriented downward) on the pad and the belt was tightene mid-plastron. No hooks were required to extend the head or h it in place. An adjustable buret clamp on a threaded rod mour