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TECHNIQUES

Herpetological Review, 2004, 35(4), 349–350.

Photographic Identification as a Noninvasive Marking Technique for Lacertid Lizards

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Color and body patterns have long been used to identify individual lacertid lizards (Schmidt-Loske 1996), but may be unsuitable for long-term studies because they vary with reproductive condition, age, and other factors (Henle et al. 1997). Elbing and Rykena (1996) found that individual *Lacerta viridis* could be recognized by patterns of head scalation, and although these scales do not vary over time (Fox 1975), Elbing and Rykena (1996) considered this method of identifying individuals to be excessively time consuming and, in some cases, difficult to use because of the small size of the scales. Recently, Steinicke et al. (2000) found photographic identification of the scales of the first four rows of ventrals to be a suitable technique for recognizing individuals of five species of lacertids (*Lacerta agilis*, *L. bilineata*, *L. viridis*, *L. vivipara*, and *Podarcis muralis*). Photographic identification of individuals by means of scale patterns is an emergent technique with a promising future, but it is necessary to examine other species before it can be considered suitable for lacertids, and other lizards generally. The method also must be improved to reduce the time required for identification, especially when many individuals are involved in the study.

We tested the suitability of using ventral scalation to identify individual *Lacerta perspicillata* (SVL = 46.8 ± 0.34, range: 40.5–54.0, N = 99; unpubl. data). From November 2000 to May 2001, we collected 53 individuals on Menorca Island, Spain. We toe clipped and took two pictures of each lizard with a digital camera (Sony Mavica®). Digital photographs (resolution 640 x 480 pixels) were enhanced in the laboratory with Microsoft Photo Editor® (brightness, contrast, and conversion from color to black and white) and printed (600 x 300 dpi). Ninety-three recaptures of the 53 toe-clipped lizards were identified from photographs using ventral characters (i.e., scales of the chin and collar area, arrangement of scales in the chest area and of the longitudinal and transverse scale rows of the trunk, Fig. 1) by two observers. Photographic identification of individuals matched identification based on toe clips in 100% of cases. All individuals were easily identified, and no apparent change in scale pattern was noted.

Our method was subsequently used in a capture-recapture study involving the same population later the same year (June–October 2001). Lizards were photographed and individuals were added to a previously assembled reference collection of pictures. Individuals collected in the field were identified via comparison with the reference file. To reduce time spent on identification, the photographic reference file was organized by sex, and then by natural amputations, scale anomalies (e.g., aberrations, asymmetries), and

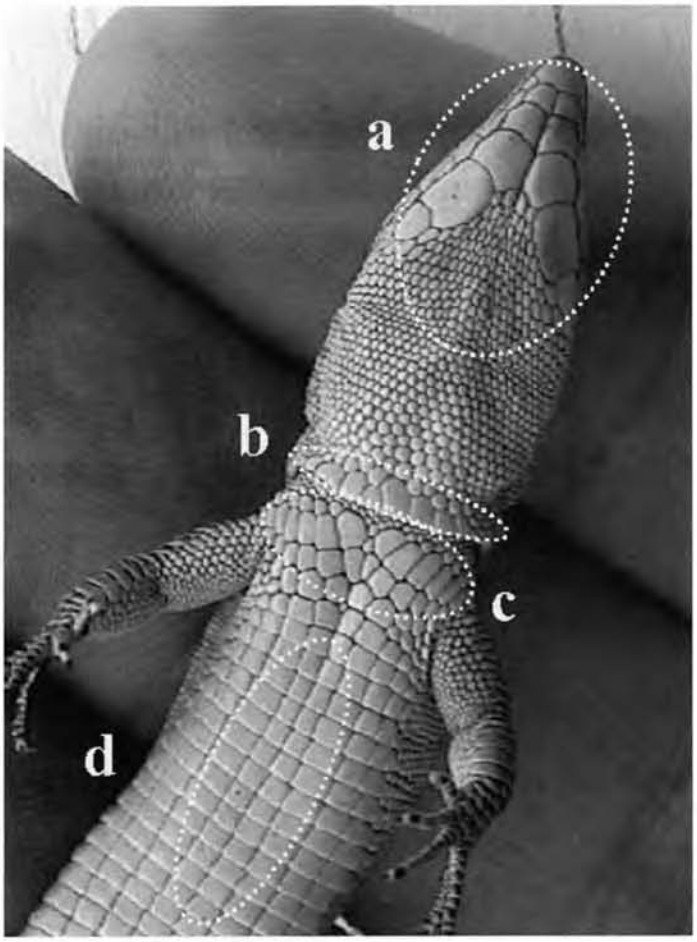

FIG. 1. Ventral characters used in the reference file: (a) chin-scale symmetry, (b) collar scales, (c) scale arrangement in chest area, and (d) position of longitudinal and transverse rows.

the ventral scale characters illustrated in Fig. 1. Consequently, the time needed to identify a lizard was reduced to < 2 min.

Our study corroborates the findings of Elbing and Rikena (1996) and Steinicke et al. (2000) and extends the use of photographic identification of lacertid scalation patterns as a means of permanent specimen identification in field studies. Digital cameras and printers greatly reduce the time and expense required for this technique, making individual recognition by means of photographic identification only slightly more time consuming and expensive than toe clipping. However, photographic identification has the advantage of being both nontraumatic and permanent, making it suitable for field research.

Acknowledgments.—We thank G. Cortázar and R. Jovani for help in the field, and "Lithica" for facilities utilized during the study. Special thanks are due to R. Jovani, R. Brown, R. Espinoza, and an anonymous reviewer for suggestions that greatly improved the manuscript. Financial support was provided by project PB98-0270 and an FPI grant by the Spanish Ministry of Science and Technology to A. Perera. This research was conducted under permit from the Balear Government (8001/2001).

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Herpetological Review, 2003, 35(4), 350–351.

A Preliminary Test and Report on the Efficiency of a New Funnel Trap for Semi-Aquatic Snakes

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Baited and unbaited glue-traps (Glor et al. 2000; Vargas 2000; Whiting 1998), funnel traps with and without floats (Casazza et al. 2000), drift fences in conjunction with pitfalls, round and square funnel traps (Christiansen and Vandewalle 2000), and habitat traps (Allan et al. 2000) have recently been proposed for the collection of reptiles and amphibians. Several collection techniques, such as collecting by hand, drum nets, fykenets, and funnel traps, have been successfully used for ecological and life history studies of aquatic or semi-aquatic snakes (Casazza et al. 2000; Fitch 1986; Shine 1986). In long-term ecological studies, funnel traps have proven to be an efficient method to systematically collect specimens, not only for aquatic species, but also for terrestrial and arboreal species (Casazza et al. 2000).

From August to October 2002, we conducted a preliminary population study on three sympatric water snakes (*Enhydris chinensis*, *Enhydris plumbea,* and *Xenochrophis piscator*) in Tamsui, Taipei County, Taiwan. Over a period of three months, two funnel trap-types were utilized in this population study to determine their effectiveness. During the three-month test, a total of 20 medium-size shrimp funnel traps (12.5 cm diameter) and 15 small-size (10 cm diameter) funnel traps modified with polyvinyl chloride (PVC) piping (referred to in the text as PVC funnel traps) were set in a private farmland composed of five ponds and adjacent paddy fields. The medium-size (12.5 cm diameter) shrimp funnel trap was 35 cm in length with a single entrance. The first funnel is situated in the entrance of the trap and the second funnel is positioned ca. 11 cm into the trap, with the funnel cones pointing inwards. A container compartment is created by the remaining part of the trap (Fig. 1). These traps were positioned horizontally and half-submerged along the shoreline of the pond and paddy field habitat

with pegs at approximately three-meter intervals.

The new PVC funnel trap is an inverted "T" in design (Fig. 1) and it has two entrances, one at each side of the bottom, with an upright vertical air circulation pipe to prevent the snakes from drowning once they become trapped. Every PVC funnel trap was constructed out of the container compartment and two entrance parts of the small-size shrimp funnel traps (10 cm in diameter). These parts were attached to a 50 cm long PVC pipe (80 mm inside diameter) fitted to a PVC T-piece connector as follows: the PVC pipe was attached to the T-piece connector, the container compartment was fitted to the upright end of the PVC pipe and the two entrance parts, fitted with funnels, were attached to the two open ends of the T-piece. Every PVC trap was kept in an upright position by tying it to a bamboo rod planted in the mud of the pond bottom. The PVC trap can also be tied to the vegetation. When the trap is set in a deep-water trapping site the air circulation pipe can be extended by attaching another pre-manufactured PVC connector. Some semi-aquatic snake studies have indicated that individuals of different gender and size utilize different water depths (Shine 1986). For that reason, every PVC trap is calibrated on the vertical air circulation pipe for measurement of water depth (Fig. 1). The PVC traps were set at approximate equal intervals from each other in the testing pond habitat. Both trap-types were baited with loaches (*Misgurnus anguillicaudatus*) to increase the capture rate.

After three months and 1636 trap-nights, we collected a total of 49 snakes of four species (Table 1) using both trap-types. Snakes were marked and released, and some snakes were trapped more than once, resulting in a total of 70 snake captures. The trap-rate was 3.42 per 100 trap-nights in the PVC traps (33 snakes in 965 trap-nights) and 5.51 per 100 trap-nights in the medium-size shrimp funnel trap (37 snakes in 671 trap-nights). Because of the water depth of the test ponds, the water depth of the PVC funnel traps was between 21 and 97 cm, much deeper than the unmodified shrimp funnel traps (< 12 cm). At this depth *E. chinensis* was more effectively trapped with the PVC funnel traps than *E. plumbea* and *X. piscator*, which might reflect some difference in microhabitat utilization among these species.

The unmodified medium-size shrimp funnel traps were effectively utilized in shallow aquatic conditions (e.g., creeks, ditches, rice paddies, and swamp habitats) in population studies of the two species of *Sinonatrix* snakes in Taiwan by the first author (Mao 2003) and in the present test. Various amphibian and reptile species and individuals of the same species, but in different age classes, might utilize different aquatic environments and depths. In deep pond environments, the PVC funnel trap proposed here allows trapping at greater depths than standard funnel traps and offers great potential for semi-aquatic snake studies.

TABLE 1. Comparison of trap rates (per 100 trap-nights) of unmodified shrimp funnel traps and PVC funnel traps. The PVC traps were used in a pond, and the shrimp funnels in swamp, creek, ditch and paddy field. *Sinonatrix* data from Mao (2003).

Species	PVC funnel trap-rate/100 nights (965 nights)	Shrimp funnel trap-rate/100 Nights (671 nights)
Enhydris chinensis (N = 32)	2.8	0.75
E. plumbea (N = 2)	0.0	0.30
Xenochrophis piscator (N = 35)	0.62	4.32
Bungarus m. multicincintus (N = 1)	0.0	0.15
Sinonatrix spp. (N = 617)	—	2.75

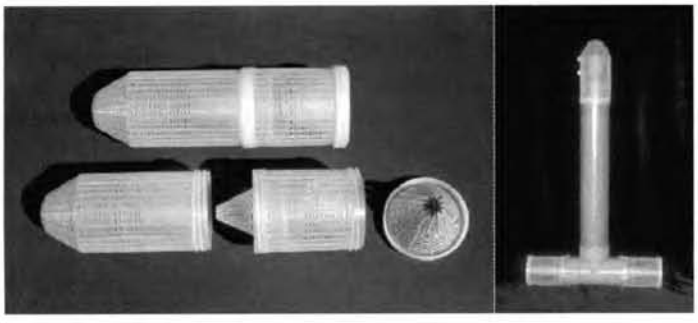

FIG. 1. Shrimp funnel trap components (left) and modified inverted "T" design (right). The upright vertical air circulation pipe allows snakes access to air.

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Bufo marinus. Male, 108 mm SVL; female, 160 mm SVL. Puerto Rico: Quebradillas, Región de San Antonio. Illustration by Fernando Vargas Salinas.

Herpetological Review, 2004, 35(4), 352–354.

Rates of Tricaine Methanesulfonate (MS-222) Anesthetization in Relation to pH and Concentration in Five Terrestrial Salamanders

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Tricaine methanesulfonate (MS-222) is commonly used to anesthetize fish and amphibians, but while studies have investigated its effects on fish, no studies have been carried out on terrestrial salamanders (e.g., family Plethodontidae). Existing general guidance recommends MS-222 baths of 500 mg/L for anesthetizing adult salamanders (Cooper 2003), however species-specific dose recommendations and pH buffering instructions are nonexistent. Neutralizing the pH of MS-222 solutions is necessary because when dissolved in water with poor buffering ability, such as tap or distilled water, MS-222 lowers the pH (Smit et al. 1977). For example, a 50 mg/L MS-222 solution prepared using distilled water has a spontaneous pH of 3.9, and a 5000 mg/L solution has a spontaneous pH of 2.8 (Ohr 1975a). Such low pH baths induce anesthetization more slowly than neutral solutions (Smit and Hattingh 1979; Ohr 1975a), and low pH solutions injure the epithelial transport systems for sodium chloride and water in leopard frogs (*Rana pipiens*; Ohr 1975b). Crawshaw (1993) recommends neutralizing the pH by adding sodium bicarbonate, but this method is cumbersome because the reaction takes some time to equilibrate, which can cause difficulty in determining the appropriate amounts of sodium bicarbonate required to attain neutrality. For example, adding 3.5 g of sodium bicarbonate to 250 ml of a 2000 mg/L MS-222 solution results in a spontaneous pH of 7.0, however after 4 hours the pH will reach >8.2 (pers. ob.).

I experimented with commercially available aqueous pH buffers consisting of dihydrogen potassium phosphate and sodium hydroxide (Fisher Scientific, catalogue numbers SB112-20, SB108-10, SB104-20) to stabilize the pH of MS-222 (Sigma, catalogue number A-5040) solutions. I investigated the relation of pH and concentration on anesthetization time in five terrestrial salamander species (*Plethodon elongatus, P. cinereus, Ensatina eschscholtzii, Batrachoseps attenuatus, Desmognathus ocoee*). I tested the null-hypotheses that there are no differences in anesthetization time among three pH levels (6, 7, 8), between two concentrations (2000 mg/L, 500 mg/L), or among species.

Materials and Methods.—Twenty salamanders (4 each of the five species) were subjected to six experimental treatments of MS-222 that included two concentrations (2000 mg/L and 500 mg/L) at three pH levels (6, 7, 8). Salamanders were submerged in the aqueous solutions and timed with a stopwatch until they were completely anesthetized. The criterion for complete anesthetization was the inability to right themselves when turned onto their backs. The experiment was stopped if after 30 minutes a salamander was not completely anesthetized. Full submersion of terrestrial salamanders may have caused stress to the animal as vigorous swimming behavior was observed for the first few seconds of anesthetizing. As an alternative method, salamanders could be partially submerged in a more shallow bath that would allow their heads to remain out of the anesthetizing solution. Following anesthetization, salamanders were rinsed with water and aquarium conditioner and allowed to recover. Salamanders were housed in an environmental control chamber at 14°C in plastic containers with clay pot shards and moist paper towels prior to and between experiments. Salamanders were fed fruit flies once per week. Aqueous solutions of MS-222 were at room temperature, and salamanders were acclimated to room temperature for 30 minutes prior to anesthetization. MS-222 solutions were prepared by mixing MS-222 powder with aqueous pH buffers. For a 2000 mg/L concentration, 0.5 g MS-222 was mixed with 250 ml of aqueous buffer. For a 500 mg/L concentration, 0.125 g MS-222 was mixed with 250 ml of aqueous buffer.

I used a three-factor repeated measures experimental design with time to anesthetization as the response variable and factors being species, pH, and concentration. Six repeated measures, representing the six treatment combinations of pH and concentration, were taken on each subject. The washout period (time between treatments) in repeated measures designs is intended to be long enough to allow subjects to fully recover, thereby preventing a previous treatment from affecting the outcome of a following treatment. I observed that after anesthetization with a 2000 mg/L solution of MS-222 at pH 7, salamanders were able to lift their heads and walk within 17 (± 2 SE) minutes. Hormonal effects of acidic (pH 3) MS-222 baths endure at least 5 h after anesthetization of leopard frogs, but these effects were attributed to the acidity of the baths not MS-222 itself (Ohr 1975b). Arterial pH of bullfrogs (*Rana catesbeiana*) exposed to neutralized (pH 7.4) and acidic (pH 3) MS-222 baths return to normal levels after one day (Ohr 1975b). No information exists on long-term physiological recovery or the effect of repeated anesthetizations. Therefore, I chose a washout period of 14 days between treatments. Repeated measures ANOVA requires the assumption of circularity, which is met when treatment effects are independent. When the assumption of circularity is not met, I report Box's epsilon adjusted p-values. A sample size of 4 individuals each of the five species (20 salamanders total) was chosen because only that number was available in the lab. I used the computer program Number Cruncher Statistical Systems™ 2000 (J. Hintz, Kaysville, Utah) to perform two, 2-factor repeated measures ANOVA tests. Factors were species and pH in one test and species and concentration in the other. I used Tukey-Kramer's multiple comparison procedure to differentiate groups of species according to their anesthetization time. The response variable (time in seconds) was normalized by the log transformation. I analyzed the effect of snout-vent length (SVL), weight, and SVL-weight ratio as potential covariates using Pearson's correlation coefficient.

Results.—The time required for treatment solutions of MS-222 to anesthetize salamanders ranged from 1 minute 9 seconds to 30 minutes (mean = 7 minutes 5 seconds) and varied by species, pH, and concentration (Fig. 1). Snout–vent length, weight, and SVL-weight ratio were not significant covariates to anesthetization time (R = 0.14, 0.01, 0.13 respectively). Significant differences were found in the ANOVAs among species, among pH levels, and between concentrations (Table 1).

Tukey-Kramer's multiple comparison procedures, which were applied to each ANOVA test separately, produced the same species grouping pattern in the pH ANOVA as in the concentration ANOVA. In both tests the two members of the genus *Plethodon*

took significantly longer to anesthetize than the other species (concentration ANOVA: MSE = 0.02, CV = 4.37; pH ANOVA: MSE = 0.03, CV = 4.37). Tukey-Kramer's multiple comparison procedures used on the pH factor showed that the significant differences among pH were between pH 8, which took less time to anesthetize, and pH 6 and 7, which took more time (MSE = 0.01, CV = 3.51). Significant interaction terms in both ANOVAs (Table 1) indicate that the manner in which species were affected by treatments of a factor were not consistent between treatments of that factor.

TABLE 1. Species, pH, and concentration had significant effects on time to anesthetization (repeated measures ANOVA), but interaction terms were also significant indicating that the degree of change between levels of a factor was not consistent among species. Tukey-Kramer's multiple comparison procedures showed pH 6 and 7 were different than pH 8 (MSE=0.01, CV=3.51) and *Plethodon* species were different than all others (MSE=0.02, CV=4.37).

Response Variable	Source of Variation	DF	MS	F	p
Time (seconds)	A: species	4	0.42	12.37	0.0001
	B(A): individual	15	0.03		
	C: pH	2	0.41	28.76	<0.0001*
	AC	8	0.03	4.63	0.0016*
	BC(A)	26	0.01		
Time (seconds)	A: species	4	0.28	16.41	<0.0001
	B(A): individual	15	0.02		
	C: concentration	1	2.74	176.52	<0.0001
	AC	4	0.11	6.92	0.0023
	BC(A)	15	0.02		

* Box's epsilon adjusted p-value.

Discussion.—Using aqueous pH buffers to prepare MS-222 solutions is a convenient way to keep the solution pH stable. Because neutralizing pH using sodium bicarbonate results in solutions that require up to an hour or more to equilibrate, it is difficult to judge the amount of sodium bicarbonate needed without laboratory tests. Using an aqueous pH buffer simplifies the pH buffering procedure. These results show that using a pH 8 buffer will have a significantly different effect (less time to anesthetization) than using either a pH 6 or 7 buffer. The question of whether to use a pH 7 or pH 8 buffer remains open. Ohr (1975a) recommended adjusting the pH to between 7 and 8 to prevent injury to the skin and prevent stress, and she noted that leopard frogs appeared calm in MS-222 baths at pH 7.4 (Ohr 1975b) and *Necturus* appeared calm when placed in MS-222 solutions at pH 7.7 (Ohr 1975a). My experiment did not quantify levels of stress, and no information exists on amphibian stress responses in solutions of pH 8 or higher. Because MS-222 baths at pH 7 induce anesthetization in a reasonable amount of time and the faster anesthetization times at pH 8 are associated with an unknown level of stress, I recommend using a pH 7 buffer unless species-specific

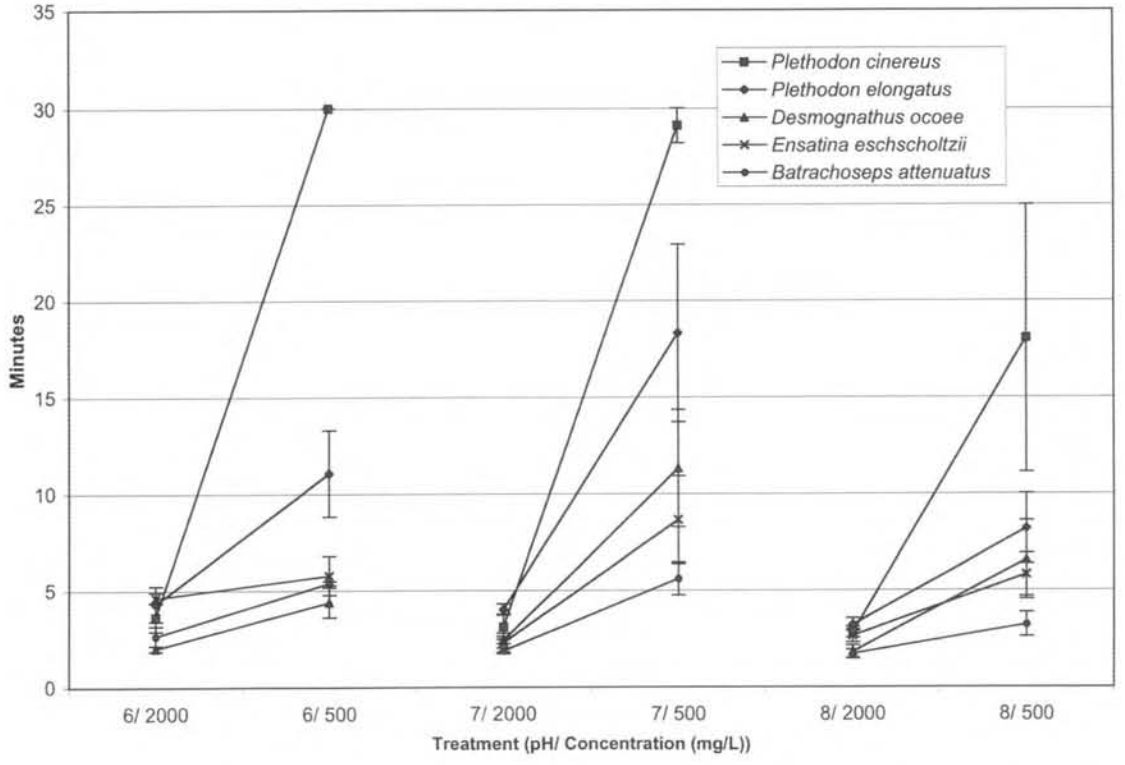

FIG. 1. Mean (± SE, N = 4) times to anesthetization are shown for five terrestrial salamander species under 6 pH/ concentration treatments. Treatments consisting of a 2000 mg/L concentration differed from those consisting of a 500 mg/L concentration (ANOVA, $p < 0.0001$). Treatments made with pH 6 and 7 aqueous buffers differed from those made with pH 8 buffer (ANOVA, $p < 0.0001$), and *P. cinereus* and *P. elongatus* differed from the other species (ANOVA, $p = 0.0001$). Standard errors are absent from one treatment because time was cut off at 30 minutes.

stress responses to other pH levels are known. Because increasing pH results in increasing the potency of MS-222, it may be necessary to decrease MS-222 concentrations if one changes their methods from unbuffered to neutral baths. For example, an unbuffered 3500 mg/L MS-222 bath normally used to anesthetize leopard frogs is lethal after being neutralized (Ohr 1975a).

As one would expect, the higher concentration of MS-222 (2000 mg/L) anesthetized salamanders faster than the lower concentration (500 mg/L). At the high concentration, all species were similarly anesthetized in under 5 minutes. At the low concentration, *P. elongatus* and *P. cinereus* took significantly longer (18 and 29 minutes respectively at pH 7) than the other species which took 6–12 minutes at pH 7. These results suggest that while 500 mg/L concentrations of MS-222 work well for some species, 2000 mg/L solutions may be needed for *P. elongatus* and *P. cinereus*. The similarity in response between *P. elongatus* and *P. cinereus* may have a phylogenetic basis due to shared physiological characteristics or may just be a result of random chance. Data on more terrestrial salamanders would be needed to support a phylogenetic hypothesis.

Acknowledgments.—I thank Darrin Doyle for his assistance in the laboratory and edits to the manuscript. I also thank John Reiss for giving his ideas and assistance and Sharyn Marks for use of her laboratory. This work was conducted in accordance with Humboldt State University Institutional Animal Care and Use Protocol #99/00.B.50.B.

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Elaphe vulpina (Fox Snake), juvenile. USA: Indiana: Tippecanoe County. Photolithograph by Will Brown.

Herpetological Review, 2004, 35(4), 354–357.

A New Method for Attaching Electronic Devices to Crocodilians

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Despite the potential importance of movement patterns to crocodilian life histories, movement, home range behavior and dispersal have received careful examination only in *Alligator mississippiensis* (Hutton 1989). Data for other species of crocodilian are deficient and stem mostly from mark-recapture studies, which have provided baseline information on many species (e.g., Gorzula 1978; Walsh and Whitehead 1993; Webb and Messel 1978; Tucker et al. 1997). However, telemetry is more suitable for clarifying theories about patterns of movement and advancing our understanding of dynamic activity budgets and seasonal patterns of habitat use by different life-history stages (Tucker et al. 1997).

Radio-telemetry has been successfully used to track *A. mississippiensis* (e.g. Joanen and McNease 1970, 1972; McNease and Joanen 1974; Goodwin and Marion 1979; Rootes and Chabreck 1993), *Crocodylus acutus* (Rodda 1984a), *Crocodylus niloticus* (Hutton 1989; Hocutt et al. 1992), *Crocodylus intermedius* (Muñoz and Thorbjarnarson 2000), *Paleosuchus trigonatus* (Magnusson and Lima 1991), and *Melanosuchus niger* (Martin and da Silva 1998). Attachment configurations for radio tags have included neck collars (e.g. ,Joanen and McNease 1970), tethering (Rodda 1984a; Rodda 1984b; Martin and da Silva 1998; Muñoz and Thorbjarnarson 2000), ingestion (Magnusson and Lima 1991), and surgical implantation (Magnusson and Lima 1991; Hocutt et al. 1992). The most successful methods in terms of longevity have been neck collars (Taylor 1984) and surgical implantation (Hocutt et al. 1992), which have enabled animals to be radio located for periods in excess of 3 and 2 years, respectively. This paper describes a new method used for attaching VHF radio tags to *Crocodylus porosus* that could easily be modified to attach other electronic devices such as satellite tags, GPS data loggers or time-depth recorders.

Captured crocodiles were physically restrained during the attachment procedure by binding the front and rear legs alongside the body with nylon webbing, and tying the animal to an aluminium ladder padded with burlap sacks. Subject animals were blindfolded with eye pads and electrical tape to reduce visual stimulation. Once restrained, the animals rarely struggled during the procedure unless provoked by loud human voices or the sound of an approaching boat. Anesthesia was not used, partly because of difficulty finding an appropriate treatment regimen that effectively and reliably sedated the animals for the intended procedure, but more importantly because of the lengthy recovery periods involved (see Loveridge and Blake 1987; Bennett 1996). Priority was given to returning the animals to the water as quickly as possible at the end of the procedure. The procedure was performed near the site of capture either onboard a small (4.5 m) boat or on the riverbank. Animals were released as close as possible to the site of capture.

Tags were attached to the enlarged nuchal scales on the dorsal

surface of the neck because the pronounced keel of these scales was conducive to the use of bone pins (Fig. 1). The tags fit between the central nuchal scales of large animals (> 3.5 m) and sat above these scales on smaller individuals. One animal had a large gash on its throat and a necrotic wound festering beneath the nuchal scales. Therefore, the tag was attached to the dorsal scales midway between the front legs.

An aluminium angle bracket was pop-riveted to the tag (Fig. 1A), which was then placed over the central nuchal scales to assess the fit (Fig. 1C). Depending on the size of the nuchal scales, the bracket could be trimmed with tin snips as required to minimize the height of the tag above the dorsal surface. The ventral surface of the tag and the bracket were sanded with emery paper to roughen the surface to aid bonding with the glue. The tag was then sprayed with 70% ethanol and allowed to dry.

Two brands of equally satisfactory glue were used: Loctite Fixmaster Underwater Repair Epoxy (<http://www.loctite.com>) or Selleys Knead It Aqua (<http://www.selleys.com.au>). Both are hand kneadable, fast setting, co-extruded epoxy repair systems that come in roll form, with the hardener encapsulated in the resin. They harden 5–10 min. after mixing to a white solid material and cure fully within an hour. Both will adhere to damp or wet surfaces and cure underwater. The glues are slightly exothermic while curing but were tested on human skin and temperatures generated were mild.

The nuchal scales were scrubbed clean with a disposable chlorhexidine scrub, rinsed with river water and dried with a clean cloth. The area was sprayed with 70% ethanol, which was allowed to evaporate. A lump of glue was placed on the ventral surface of the tag, which was then placed between the central nuchal scales. The tag and glue were molded to remove any air pockets and minimize the tag's profile above the dorsal surface while leaving the outside lateral edges of the central nuchal scales exposed to enable the placement of bone pins (Fig. 1B).

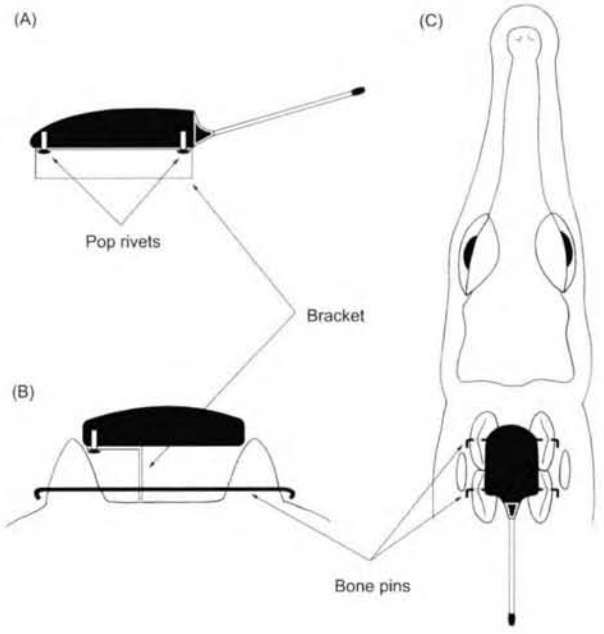

FIG. 1. Placement and orientation of the radio tag, bracket and bone pins on the nuchal scales. The attachment was further augmented with glue, which bonded sufficiently well to the tag and bone pins that the bracket is now considered redundant.

The bone pins used were 31 cm, 1.6 mm diameter, stainless steel Kirschner wires (K-wires), which were cut in half and secured directly into the chuck of a cordless drill. They were then sprayed with 70% ethanol for sterilization. Two pins were used, one through the anterior central nuchal scales and a second through the posterior central nuchal scales (Fig. 1C). The K-wires had a trocar spike at each end, which enabled them to drill directly through the osteoderms and bracket without pre-drilling any holes, but the process was fairly slow. Care was required to ensure the orientation of the bone pins was horizontal and that they penetrated only the raised keel of the scales and the bracket (Fig. 1B). Once the bone pins protruded through the osteoderms they were bent with pliers, to stop them from pulling through, and trimmed with wire cutters (Fig. 1B, C). The area was sprayed again with 70% ethanol, which was allowed to evaporate. Additional glue was then placed around the tag and molded to provide smooth contouring, and totally encase and seal the central nuchal scales, bone pins and the lower half of the tag.

Ten tags were attached using brackets but, because it was thought that the glue bonded sufficiently well to the tag alone, no bracket was used for 6 other attachments (Table 1). The length of time a tag stayed attached to a crocodile was similar, irrespective of whether a bracket was used (15– > 412 days) or not (132– > 370 days), and I now consider the bracket to be superfluous. Elimination of the bracket not only reduced the materials required but also the time taken to attach a tag. Time taken to attach a tag ranged from 60 min. (bracket and inexperience) to about 30 min. (no bracket and experience).

Tag attachment without a bracket was a much simpler procedure. The nuchal area was prepared as before. Holes for the bone pins were pre-drilled through the keel of the nuchal scales with a sterilized drill bit, which was much faster than trying to drill holes using the trocar spike at the end of the K-wires. Also, aligning the bone pins was much easier. After the bone pins were placed through the scales, they were bent and trimmed as before, and the area was then sterilized with 70% ethanol. Once the ethanol evaporated, the glue and tag were placed over the central nuchal scales and molded into place so that the glue enclosed and sealed the bone pins and scales, and held the tag in place.

Every effort was made to use as sterile a procedure as possible, given the limitations of working under field conditions. Because bone pins were used, a single phrophylactic dose of a broad-spectrum antibiotic, oxytetracycline hydrochloride (OTC), was administered by intramuscular injection as a precaution prior to commencement of the attachment procedure. Dose rates were calculated by allometrically scaling the therapeutic dose recommended by the manufacturer for placental mammals, to that for a generic reptile, using the methods described by Pokras et al. (1992) or Sedgwick and Borkowski (1996). OTC has the added advantage of being a suitable biomarker (see Coles et al. 2001). It is worth noting that crocodiles, in common with other reptiles and non-mammalian vertebrates, have a renal portal system. Therefore, it is prudent to inject any medications, especially nephrotoxic drugs, into the anterior half of the animal to avoid the renal portal system (Jenkins 1996).

Tags weighed 140 g but, when combined with glue, pins and bracket, the mass of the assembly increased to about 500 g. In

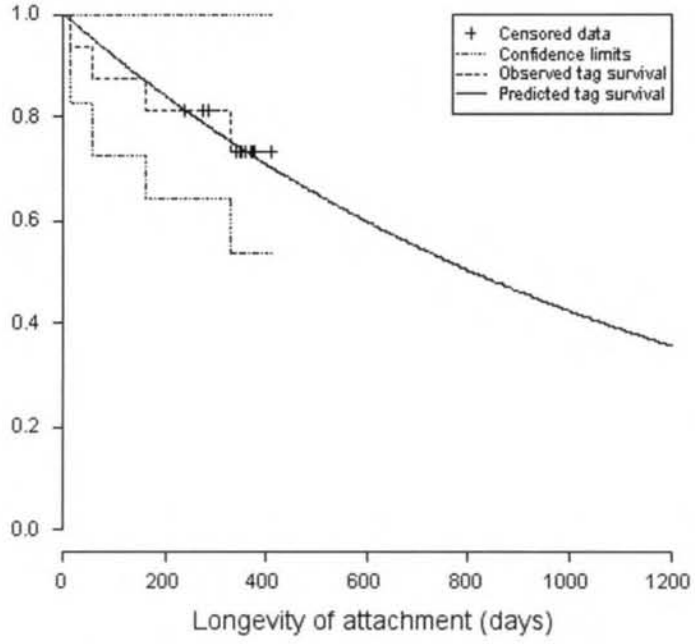

FIG. 2. Exponential survival function fitted to longevity of attachment data. The dashed line is the observed tag attachment time with each step down representing a detachment event. The crosses represent censored data, that is, individuals where the tag was still attached and operational at the final observation. The solid line is the tag attachment time predicted by fitting an exponential function to the observed data.

general, tags should weigh < 3–5 % of body mass to avoid adverse effects (Kenward 2001). Therefore, using this method with current tag specifications, animals would need to be > 17 kg or about 1.8 m total length. The greatest confirmed longevity of attachment (LOA) achieved during the study was > 412 days and most tags remained attached for > 340 days (Table 1). The study ended in May 2003, however, aerial crocodile surveys flown in July 2003 sighted three tagged animals, confirming one tag had remained attached for > 637 days and two others for > 420 days. Four tags (25%) are known to have detached during the study: three detached naturally in submerged snags and one when an animal escaped from a cage trap (Table 1). Dislodgement of a tag is most likely during a flight response when an animal is startled, especially when it is among thick or fallen vegetation, whether submerged or on the bank.

A simple mean LOA would not provide a meaningful estimate of expected attachment time because most tags were still attached and operational at the end of the study (= censored data, see McCallum 2000; Crawley 2002). Therefore, survival analysis on LOA was performed to estimate the mean time to failure using the survival package within R statistical software (ver. 1.6.2, Ihaka and Gentleman 1996). No evidence was found that the risk of detachment increased with attachment time (Weibull distribution, scale = 1.33, $P = 0.5$ for H_0 scale = 1). Therefore, it was assumed that the risk of detachment was constant throughout life, and an exponential survival function was fitted to the data using parametric regression (McCallum 2000; Crawley 2002). The mean time to failure was estimated to be 1164 days. The precision of the estimate was low, having a 95% CI of between 437 and 3103 days, because most of the data were censored with few failures occurring during the study period (Fig. 2).

Crocodile 185 was re-captured after 370 days and the tag removed to evaluate possible deleterious effects of the attachment procedure. The skin appeared healthy with only a slight loss of pigmentation (see Kirshner 1985) and there was no infection or necrosis visible in the underlying tissue. Skull dimensions, total length and body mass had all increased suggesting the attachment

TABLE 1. Longevity of attachment (LOA) achieved for 16 animals tagged during the study.

Crocodile	Sex	Total length (m)	Body mass (kg)	Date attached (dd/mm/yy)	Bracket (Y/N)	LOA at last fix (days)	Comments
146	M	2.65	59	15/10/2001	Y	15	Tag detached escaping trap
147	M	2.09	26	22/10/2001	Y	241 +	
350	M	2.13	32	23/10/2001	Y	277 +	
164	F	2.72	76	25/10/2001	Y	412 +	
183	F	3.08	103	11/04/2002	Y	281–384	Tag detached in snag
184	M	3.25	91	22/04/2002	Y	377 +	
186	M	3.27	141	24/04/2002	Y	375 +	
188	M	3.53	151	07/05/2002	Y	361 +	
189	F	2.72	82	07/05/2002	Y	360 +	
190	M	2.63	62	09/05/2002	Y	49–67	Tag detached in snag
185	F	2.74	76	24/04/2002	N	370	Tag removed 29/04/2003
191	M	4.34	337	12/05/2002	N	287 +	
192	M	3.12	111	16/05/2002	N	352 +	
193	M	3.17	116	21/05/2002	N	348 +	
194	M	3.07	103	25/05/2002	N	132–195	Tag detached in snag
195	M	2.53	62	26/05/2002	N	341 +	

+ Tag still attached and operational at last fix

had not adversely affected growth or body condition. Furthermore, all females tagged during the study moved to nesting habitat during the wet season and one was detected near a recently constructed nest. Therefore, attachment of the tags did not appear to have interfered with courtship, mating or nesting behavior. On the basis of this evidence, I consider the technique to be relatively benign.

In summary, this technique provides a reliable medium to long-term method for attaching small electronic devices to crocodilians. It has some advantages over other successful methods of tag attachment. First, it does not constrict an animal's neck as would a collar and it is not as invasive as surgical implantation. Also, signal propagation is better than with an implanted tag, which improves detectability. Examination of underlying tissue on one animal 370 days after attachment suggests that the method is relatively benign. There are a number of ways the technique could be refined. Ideally, the tag should have as low a profile as possible above the dorsal surface of the animal. In hindsight, it would have been preferable to design a narrower tag that would fit between the central nuchal scales on a greater size range of animals to reduce the overall profile of the tag assembly above the dorsal surface. However, there is a compromise in that the tag itself would have a higher profile. Additionally, the flexible whip antenna could be orientated vertically without greatly increasing the risk of detachment, which may improve signal propagation and reception (see Kenward 2001). The use of alternative materials may also be advantageous. For example, bone pins made from titanium or Delrin plastic (<http://plastics.dupont.com>) are probably more inert than stainless steel. It would be well worth testing the use of a less dense, flexible polyurethane glue such as Sikaflex-291 (<http://www.sika-industry.com>), which would reduce the overall weight of the tag assembly. However, curing times for this product are lengthy and would need to be accelerated to be of practical use in the field.

Acknowledgements.—This work was funded by the Department of Conservation and Land Management and Lotterywest through a Gordon Reid Foundation grant to the Save Endangered East Kimberley Species (SEEKS) community group of Kununurra. In addition, I would like to thank Nick Gales, Rick Read, Rob Day, Paul Clarkson, Sarah Brett, Grahame Webb and Gordon Grigg for useful discussions on potential methods and materials for attaching transmitters. Thanks also to Jane McRae for drawing the illustrations in Fig. 1, Hamish McCallum for advice on statistical procedures and Charlie Manolis for reporting tagged animals sighted during aerial surveys.

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Herpetological Review, 2004, 35(4), 358–359.

A Novel Technique for Capturing Arboreal Geckos

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When conducting research on lizards it is often necessary to capture individuals unharmed. Popular methods for the capture of arboreal lizards include hand capture, noosing, using rubber bands as projectiles (Simmons 1987), chasing lizards into mesh barriers (Patterson 1998), attracting individuals to baited traps (Durden et al. 1995; Zani and Vitt 1995), or fishing for them with baited lines (Krysko 2000; Strong et al. 1993). Other methods include using wire hooks to pull lizards from refugia (Bedford et al. 1995), glue boards for passive capture (Bauer and Sadlier 1992), or sticky poles for active capture (Durtsche 1996).

Any capture technique has inherent problems (Krysko 2000), but for the capture of small, skittish, and fast-moving lizard species, such as geckos that perch high on building walls, the techniques listed above make capture without harm extremely difficult and, in many cases, inappropriate. The most suitable of these techniques is noosing, but often the lizard flees at the sight of the approaching pole, evades the noose, or bites the noose pulling it closed (pers. obs.). Furthermore, the thread of the noose has to be thin, making it difficult to see more than 3-m away and difficult to keep steady. Using a baited line poses similar problems to using a noose.

To overcome the problems of safely catching arboreal geckos from buildings, I describe a novel technique I developed to capture native and introduced geckos in Mauritius using a laser pointer.

All geckos rely on their vision to capture predominantly insectivorous prey and are, in general, opportunistic feeders, attacking any small moving object within range. A small dot projected onto a wall from a laser pointer within view of a gecko will entice the lizard to give chase and repeatedly lunge and bite at the dot. The dot can then be moved down the wall to a point permitting capture, generally at chest height, close to an awaiting hand. To keep the gecko moving it is necessary to keep the laser dot within 5–10 cm of the gecko's head.

Lasers are grouped into seven classes (1, 1M, 2, 2M, 3R, 3B, and 4), the higher the class the greater risk of laser radiation hazard. Most commercially available laser pointers belong to Class 1 or 2. The output power of a Class 1 laser is such that exposure of the eye to the beam will not cause damage and is, therefore, considered eye safe. The output power of a Class 2 laser is higher and prolonged eye exposure is potentially harmful, although a person's natural involuntary response, such as the blink reflex and/or head aversion is quicker than the maximum permissible exposure time, therefore avoiding damage to the eye. The International Electrotechnical Commission (IEC 60825–1 2001) and British Standards (BS EN 60825–1 1994) state that only Class 1 or 2 devices should be used in unsupervised areas. Of course, when using any type of laser pointer, do not shine the laser beam into the eyes of any person or animal. The risk of shining the beam of the laser into the eyes of a gecko is easily avoided by continually moving the laser away from the target animal. Catching geckos from reflective surfaces, such as glass windows, should be avoided. Researchers, should consult regional laws and regulations before undertaking fieldwork using laser pointers because some countries have laws associated with possession and use of laser pointers.

The efficiency of the technique is illustrated for four species of nocturnal gecko and the daytime capture of two diurnal species (Table 1). The laser pointer was only used for geckos that could not be caught by noose or by hand. Individual geckos were not captured more than once for this trial. The utility of this method, therefore, is untested for experimental designs requiring multiple captures of the same individual. More than 90% of all geckos pursued the laser dot and almost 80% of these were brought to a point of capture. Five geckos were so intent on eating the laser dot that they chased the dot down onto the ground (up to 11 m) and two were coaxed onto the palm of my hand. Of the 19 geckos that were not brought to a point of capture, seven showed no response to the laser dot, one ran off, six terminated their pursuit after chasing the laser dot (one after pursuing it for 8 m), and five either encountered and ate an insect or were chased off by another gecko whilst following the laser dot.

Nocturnal geckos responded similarly in chasing the laser dot over unlit (51%) and lit (49%) walls and over smooth (cemented/bricked [58%)]) and rough (old stone [42%]) walls. All diurnal geckos were found on shaded walls. It is doubtful whether geckos would be able to see the laser dot in full sunlight. Eighty six percent of diurnal geckos were found on smooth surfaces, so it is difficult to determine whether wall texture had an effect on efficiency of laser use.

This technique is quick to use, inexpensive, requires minimal equipment, and aids in the capture of individuals from high, unreachable places without harm. The use of the laser pointer has also been successful in enticing adult *Phelsuma ornata* down the trunks of coconut trees (data not included here), indicating that its use may not be restricted to relatively flat surfaces, such as walls.

TABLE 1. Number of each lizard species indicating the percentage of adults (rounded to nearest whole digit), the efficiencies of the laser technique, and distances they moved.

Species	N	Geckos chasing laser dot (%)	Brought to hand capture (%)	Mean distance (m) moved by responsive geckos (SD, maximum)
Gehyra mutilata	9	78	78	4.3 (1.1, 6)
Hemidactylus brookii	7	100	86	6.1 (2.3, 9)
Hemidactylus frenatus	58	97	85	4.4 (2.2, 11)
Hemiphyllodactylus typus	3	67	33	3.5 (0.7, 4)
Phelsuma cepediana	3	67	67	3.5 (0.7, 4)
Phelsuma ornata	14	86	71	3.3 (1.7, 6)
Total or Mean (%)	94	92	80	4.3 (2.1, 11)

This method is also likely useful in facilitating the capture of other small insectivorous lizards. Another potential use could be in social experiments wherein an individual is enticed into the territory of another to test behavioral interactions.

Acknowledgments.—This technique was developed under a NERC grant NER/S/A/2001/05996 and a grant from the Zoological Society of Wales. I thank S. Harris and C. Austin for their comments and improvements of the manuscript.

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Herpetological Review, 2004, 35(4), 359–361.

A New Method for Preparing Preserved Hemipenes of Lizards for Scanning Electron Microscopy

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Lizard hemipenis morphology has a long history of use in taxonomy (e.g., Arnold 1986a, b; Branch 1982; Cope 1895, 1896). In other taxa the morphology of intromittent organs has also provided valuable insight into the mating strategies of a species (Arnqvist 1997; Arnqvist et al. 1997; Dixson 1987; Eberhard 1985; Patterson and Thaeler 1982). Thus, detailed studies of hemipenis morphology can be useful in a number of research fields.

Investigations of hemipenis morphology are best made on freshly prepared material, as this minimizes the potential for artifacts and allows the natural shape of the organ to be studied. Unfortunately, fresh material is not always readily available and researchers often must rely on material from museum specimens.

Squamate hemipenes are particularly difficult to study once preserved, as they lie in an inverted state within the base of the tail when not erect. Consequently, to observe their structure and surface features, it is necessary to evert these organs. This is best done at the time of preservation because fixation tends to harden tissue, making it difficult to evert fixed hemipenes without causing damage to their structure. As a result, it is common for researchers to dissect inverted organs of preserved specimens *in situ* to investigate their surface features (e.g., Arnold 1973; 1986a; Böhme 1988; Dowling and Savage 1960). The disadvantage of this method is that it provides little insight into the overall structure of the everted organ.

More recently, Pesantes (1994) described a method for softening fixed snake hemipenes by soaking the material in a 2% solution of potassium hydroxide (KOH) for 3 d. We tested this technique on eight species of New Zealand geckos but found that it led to tissue damage. In this paper we report these results and describe a new technique for softening fixed hemipenes that is easy to use and causes little tissue damage.

As part of a comparative study on interspecific variation in hemipenis morphology of New Zealand geckos (Gekkonidae), we initially followed Pesantes' (1994) method to soften the inverted hemipenes of 16 museum specimens belonging to eight gecko species that had been fixed in formalin. The condition of the fixed specimens was compared with the hemipenes of six *Hoplodactylus maculatus* that had been preserved in ethanol and did not require any treatment prior to eversion because the hemipenial tissue had remained soft and pliable.

The preparation history of the 16 museum specimens used in our study was uncertain, but all had been fixed in formalin (concentration and duration unknown) and stored in 70% ethanol. The specimens had been stored for 19–35 yr. Hemipenes were first dissected in their entirety from the base of the tail (following Arnold 1986a). Samples were rehydrated from 70% ethanol to distilled water in decreasing concentrations of ethanol (50 and 30%), by soaking the tissues for a minimum of 1 h at each concentration. The hemipenes were then softened using KOH (Pesantes 1994). We found that samples (N = 6) that had been soaked in a 2% solution of KOH for 3 d either partially or totally disintegrated. Therefore, we modified this method and obtained best results when the hemipenes were soaked in a 1% solution of KOH for 5–11 h. Following softening, hemipenes were rinsed in 0.05N HCl for 10 min and soaked overnight in distilled water. Specimens were then everted in distilled water using round-tipped forceps and a blunt probe to push the lobes inside out. It is usual for researchers to inflate everted hemipenes to obtain maximal turgidity (e.g., Böhme 1988; Glaw et al. 1999; Myers and Cadle 2003; Zaher and Prudente 2003). However, the degree of inflation that naturally occurs is unknown and it is thought that organs are often over-inflated (Arnold 1986a). In addition, it is difficult to standardize the amount that an organ is inflated. Consequently, for interspecific comparisons in which the surface features rather than the size of the organ are of primary interest, it is preferable to avoid this procedure if possible. Because the hemipenes of the species studied were very small, the organs could be fully everted with relative ease, allowing the surface features to be observed without inflation.

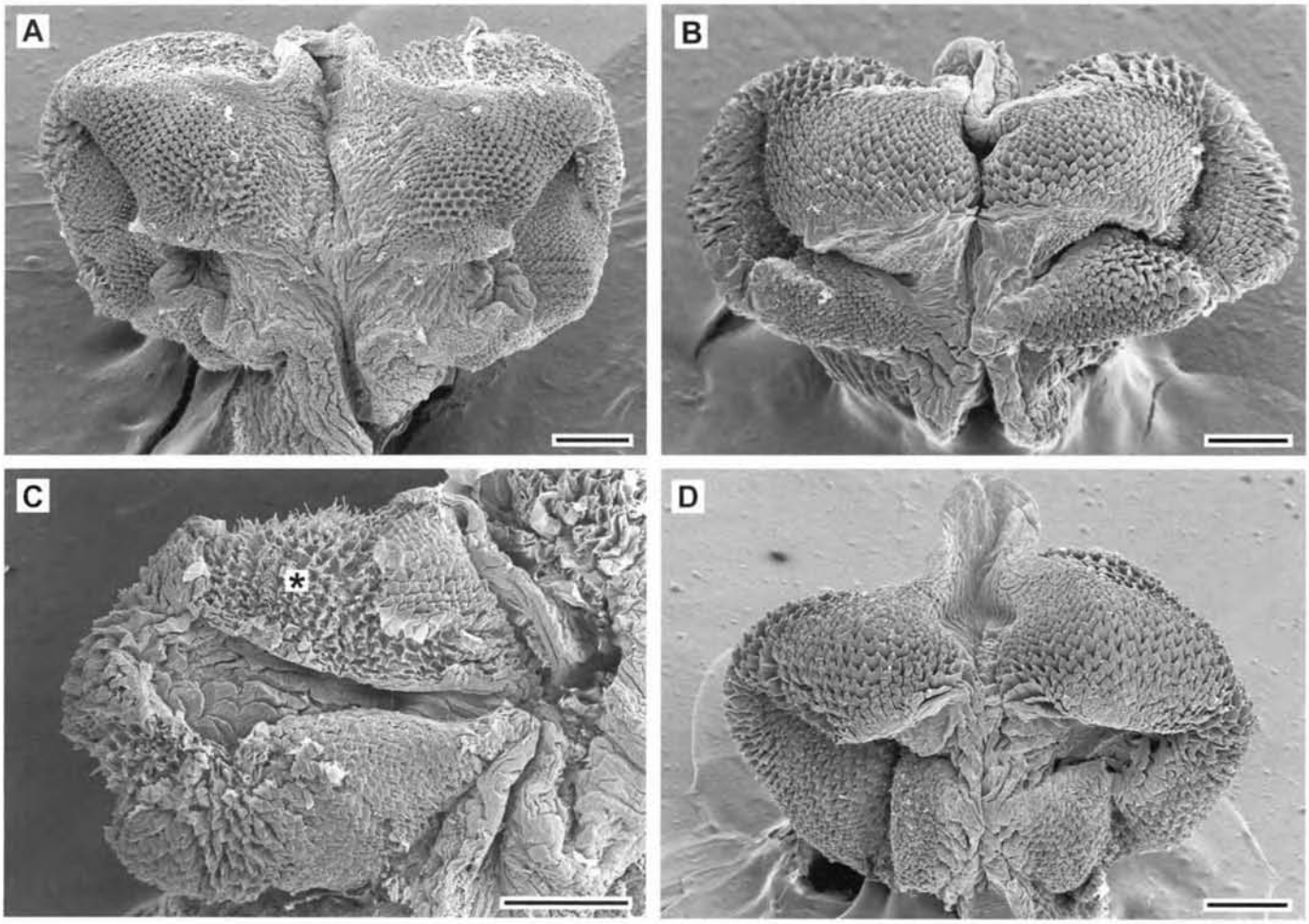

FIG. 1. SEM micrographs of the sulcal surface of hemipenes of *Hoplodactylus maculatus* (Gekkonidae) prepared using different treatments: (A) specimen softened in 1% KOH; (B) untreated ethanol-stored sample; (C) single lobe of hemipenis damaged when softened in 1% KOH, note loss of calyces (*); (D) saponin-prepared sample. Scale bars = 500 µm.

Following eversion, the hemipenes were fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1M sodium cacodylate for 3 d. Samples were then prepared for scanning electron microscopy (SEM) using the following procedure: washed overnight in 0.1M sodium cacodylate buffer, stained in 2% osmium tetroxide (OsO_4) in distilled water for 2 d, soaked in distilled water for 2 h, dehydrated through an ethanol series (30, 50, 70, 80, 90, 95, and 100%) for a minimum of 2 h at each concentration, left overnight in fresh absolute ethanol, placed in a four-step increasing concentration gradient of amyl acetate in ethanol (25, 50, 75, and 100%) for a minimum of 2 h at each concentration, left overnight in pure amyl acetate, critical point dried with liquid CO_2, mounted on aluminium SEM stubs using conductive carbon paint, and sputter-coated with ca. 40 nm gold/palladium. Hemipenes were viewed using a LEICA S440 scanning electron microscope at accelerating voltages of 10–20 kV.

The use of KOH to evert fixed hemipenes gave variable results. Of the 16 samples, four were everted in excellent condition (Fig. 1A), equal to that of unfixed specimens that had been stored in ethanol and did not require any treatment prior to eversion (Fig. 1B). However, for the remaining samples the KOH treatment resulted in partial or complete loss of the surface layer of calyces (Fig. 1C). Glaw et al. (1999) also found that KOH was sometimes too harsh for delicate organs. Accordingly, we concluded that a milder softening agent was required.

Detergents have been used previously to partially recover formalin-fixed material (Humason 1962). Maupin and Pollard (1982) demonstrated that the permeability of cell membranes to fixatives and stains could be improved by soaking the tissue in saponin, a plant glycoside with detergent-like properties that is extracted from the bark of *Quillaja*. We tested whether saponin (Sigma Cat. # S4521) could soften formalin-fixed hemipenes and enable us to evert them more easily and with less damage than we experienced with the KOH treatment.

We dissected and rehydrated hemipenes from 22 specimens belonging to 10 gecko species using the methods described previously and then soaked them in a 1% solution of saponin in distilled water. The samples were checked at 15-min intervals to detect the best time for eversion using a blunt probe to test the material for optimal softness and pliability. We found that the organs regained sufficient flexibility for eversion after 1–2 h in the saponin solution. The hemipenes were then everted, rinsed 3–5 times in distilled water over a 30-min period and prepared for SEM as described above.

Saponin treatment yielded excellent results in 16 out of 22 samples (Fig. 1D), comparable to the most successful KOH-pre-

pared specimens and the ethanol-stored samples. Although the minimum soaking time required to sufficiently soften the material varied between samples (presumably a function of their original collection/storage regime), there appeared to be no risk of over softening the tissue and thus losing delicate surface features. Only in six samples did saponin fail to soften the organs sufficiently to evert them. In these instances the tissue had been left in the solution for 2.5 h and appeared to have regained sufficient flexibility for eversion. However, upon attempting eversion we found that the material crumbled, as the tissue inside was still hard and brittle. Longer soaking times may have been required for these samples, or some samples simply may not be reclaimable using this method.

The use of fixed material to investigate the surface features of squamate hemipenes may always be, at best, a salvage operation. This is often necessary because of the lack of suitably preserved specimens. We found that saponin is a highly effective softening agent of formalin-fixed material, as not only are preparation times relatively short, but the treatment is also very mild to the tissue. Consequently delicate structures on the surface of the organ are retained throughout processing. It is possible that other solutions with detergent-like properties may prove successful in salvaging similarly preserved tissues.

Acknowledgments.—We are very grateful to R. Coory for permission to dissect preserved specimens from the Museum of New Zealand Te Papa Tongarewa, and T. Whitaker and B. Thomas for the provision of ethanol-stored specimens of *Hoplodactylus maculatus*. We also thank J. Briskie, H. G. Dowling and an anonymous reviewer for critical comments on the manuscript. This research was funded by a Brian Mason Trust grant and the Shirtcliffe Fellowship. A. Todd was supported by a Foundation for Research, Science and Technology Top Achiever Doctoral Scholarship.

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HERPETOLOGICAL HUSBANDRY

Herpetological Review, 2004, 35(4), 361–364.

A Comparison of Vitamin D-Synthesizing Ability of Different Light Sources to Irradiances Measured with a Solarmeter Model 6.2 UVB Meter

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Recognition of nutritional metabolic bone disease (= nutritional secondary hyperparathyroidism) in herpetocultural collections in recent decades has led to an interest in measuring ultraviolet B (UVB) radiation (280–315 nm) in natural light and in vivaria illuminated with artificial lamps. UVB facilitates photoisomerization of 7- dehydrocholesterol (pro D_3) to previtamin D_3 (preD_3) which in turn is thermally isomerized to vitamin D_3 (vitD_3) (Chen 1999;

TABLE 1. Demonstration of reciprocity between the UVB dose and the percent of product formation in ampules of 7-dehydrocholesterol between 30 minutes and 120 minutes. The UVB source was a Westinghouse FS 20/T12 fluorescent lamp. Irradiance was measured with a Solartech 6.2 meter.

IRRADIANCE ($\mu W/cm^2$)	EXPOSURE TIME (min) (mJ/cm^2)	DOSE	% PRODUCT SYNTHESIZED
142	30	255	34.42
70	60	252	34.20
35	120	252	34.14

TABLE 2. Various characteristics of natural light in Boston, Massachusetts (USA) (42°N) at different seasons and different times of the day. Irradiances were measured with a Solarmeter 6.2 radiometer (Solartech, Inc., Harrison Township, Michigan 48045). The regression equation relating the % of photoproducts formed to the dose is presented for each season. Ampules used to assess vitamin D-synthesizing ability of the light were exposed for 1 hour.

DATE; TIME (EST)	IRRADIANCE ($\mu W/cm^2$)	DOSE (mJ/cm^2)	% PRODUCT SYNTHESIZED
21-Jan-2003			
1100	42	151	0.17
1200	56	202	0.19
1300	52	187	0.25
1400	37	133	0.15
1500	20	72	0.06
%Prod = 0.001 (Dose) - 0.020 ($r^2 = 0.8322$)			
3-Mar-2003			
1130	120	432	0.66
1230	132	475	0.98
1330	124	446	0.85
1430	97	349	0.48
1530	50	180	0.16
%Prod = 0.003 (Dose) - 0.351 ($r^2 = 0.9249$)			
26-Jul-2003			
1100	260	936	5.2
1200	262	943	6.8
1300	275	990	7.4
1400	240	864	6.8
1500	215	774	6.5
1600	130	468	2.0
%Prod = 0.009 (Dose) - 1.730 ($r^2 = 0.7650$)			
24-Sep-2003			
1100	140	504	2.0
1200	180	648	2.7
1300	215	774	3.5
1400	185	666	3.2
1500	160	576	2.0
1600	95	342	1.2
%Prod = 0.006 (Dose) - 0.803 ($r^2 = 0.9274$)			

TABLE 3. Characteristics of four UVB emitters. Irradiances were measured with a Solarmeter model 6.2 radiometer (Solartech, Inc., Harrison Township, Michigan 48045). The regression equation relating the % of photoproducts formed to the dose is presented for each source. Ampules used to assess vitamin D-synthesizing ability of the UVB sources were exposed for 2 hours. The temperatures adjacent to the distances listed for the Westron 160 W spot were those measured after 2 hours.

LAMP DISTANCE (cm)	IRRADIANCE ($\mu W/cm^2$)	DOSE (mJ/cm^2)	% PRODUCT SYNTHESIZED
BLACKLIGHT 350 BL			
11	25	180	3.37
23	13	94	1.34
34	7	50	0.91
46	4	29	0.45
%Prod = 0.019 (Dose) - 0.162 ($r^2 = 0.9775$)			
REPTISUN 5.0			
11	36	259	1.39
23	16	115	0.54
34	9	65	0.27
46	5	43	<LD*
%Prod = 0.006 (Dose) - 0.116 ($r^2 = 0.9997$)			
ESU REPTILE DESERT 7% UVB			
11	53	382	1.79
23	25	180	0.68
34	14	101	0.35
46	8	58	<LD*
%Prod = 0.005 (Dose) - 0.209 ($r^2 = 0.9969$)			
WESTRON 160 W SPOT			
30 (45°C)	173	1246	4.39
42 (38°C)	92	662	2.68
88 (29°C)	24	173	1.07
127 (26°C)	12	86	0.82
%Prod = 0.003 (Dose) + 0.562 ($r^2 = 0.9992$)			

* less than lower limit of detection

Holick 2004). The irradiance of UVB is related to the rate of $preD_3$ production and hence indirectly to the rate of $vitD_3$ synthesis.

Spectroradiometers can accurately measure the irradiance of UVB from a light source. However, they are expensive and relatively difficult to work with. Hand-held broadband radiometers are less expensive and easy to use but may indicate irradiances that are significantly different from the actual values. Gehrmann et al. (2004) examined three types of broadband radiometers and showed that the meters indicated different levels of irradiance from the same light source. The determination of the vitamin D_3-photosynthesizing ability of a light source can serve as an independent measure of UVB irradiance. Thus, the percentage of photoproducts formed from $proD_3$ after exposure to the light for a specified time period allows for the comparison of irradiances from the different meters.

We recently became aware of an inexpensive broadband UVB meter manufactured by Solartech, Inc. (Harrison Township, Michigan 48045) as model no. 6.2, reading UVB between 280 and 320 nm and with a resolution of 1 $\mu W/cm^2$. In order to facilitate comparisons with the three meters described by Gehrmann et al. (2004) (Gigahertz-Optik, Inc. [Newburyport, Massachusetts 01950], UVP, Inc. [Upland, California 91786], and Spectronics Corp. [Westbury, New York 11590]) we correlated irradiances and associated doses from natural sunlight and various lamps with in vitro $vitD_3$-synthesizing ability.

Measurements of UVB in natural light at different times of the day were made in Boston, Massachusetts during the four seasons of 2003. UVB irradiance readings for the 1 hour during which $proD_3$-containing ampules were exposed were recorded at the start, middle, and end of the hour and then averaged. Readings were made at a solar angle of 80°. Irradiances from the three 20-watt fluorescent lamps were recorded at various distances below the midpoint of the lamp length; the meter was slowly moved about at this location to achieve the maximum reading. Irradiances were recorded at various distances below the center of the light circle produced by the Westron mercury vapor lamp. $ProD_3$ ampules were placed at these positions and exposed for 2 h to maximize conversion.

After exposure to the light source, the boron-silicate ampules that had contained 50 μg of $proD_3$ dissolved in one ml of ethanol

were analyzed by High Performance Liquid Chromatography (HPLC) for $proD_3$ and UVB-induced photoproducts ($preD_3$, tachysterol, and lumisterol) and $vitD_3$. A Waters 501 HPLC pump was used in conjunction with a Waters 490E multiwave detector set at 260 nm. The column was Econosphere silica, 5 µm, 250 x 4.6 mm (Alltech Associates, Inc, Deerfield, Illinois). The mobile phase was 8% ethyl acetate in hexane with a flow rate of 1.8 ml/min. Three replicates per ampule were analyzed and the percent of photoproducts and $vitD_3$ synthesized was calculated (see Gehrmann et al. 2004 and Webb et al. 1988 for details of the HPLC procedure).

We wished to verify that comparisons of photoproduct formation between the natural light ampules exposed for one hour and those exposed to the lamps for two hours were valid. The reciprocity law (Parrish et al. 1978) states that the UVB effect on photoproduct formation is not from irradiance *per se* (i.e., rate of energy delivery as watts), but rather the total energy (joules) delivered during a given time period which is the dose [dose (mJ/cm^2) = irradiance ($\mu W/cm^2$) x time (seconds) ÷1000]. We selected three different exposure times (30, 60, and 120 minutes) and adjusted the irradiance from a lamp, by changing the distance, such that the delivery dose was the same for all three periods. If the law was applicable to this study, the percent of photoproducts formed in the ampules should be the same for the three exposure times.

Results (Table 1) demonstrate the validity of the reciprocity law and justify comparisons of doses and associated photoproduct synthesis between ampules exposed to natural light for one hour with those exposed to various lamps for two hours. This idea is embodied in the regression equations relating the percent of photoproduct formed to the UVB dose (not the irradiance) that are given in Tables 2 and 3. Table 2 shows the irradiances of UVB in natural light in Boston measured with a Solartech 6.2 meter, and the associated doses, and relates them to the percent of photoproducts formed in ampules after one hour of exposure. Daily and seasonal trends are evident. Table 3 contrasts the UVB irradiances at different distances from four lamp types commonly used in herpetoculture and indicates their ability to produce $vitD_3$ photoproducts in ampules exposed for two hours. It is evident that the same irradiance and associated dose from different lamps can produce different quantities of photoproducts. Not all wavelengths within the UVB band are equally effective in producing $preD_3$ from $proD_3$. The greater the percent photoproduct formed at a given dose, the greater the concentration of UVB energy clustered around the most effective wavelength of 295 nm. This is clearly demonstrated by the Westinghouse FS 20 UVB lamp in Table 1. The percent of photoproducts formed is considerably higher than for the other light sources described in this article because of the high concentration of UVB close to 295 nm.

Evaluating the significance of $vitD_3$-synthesizing potential for herpetocultural purposes remains largely unexplored. Systematic studies showing the effect of latitude on the ability of natural light to form photoproducts in ampules could serve as an estimate of UVB requirements for various species in captivity taken in the context of their natural history and habitat preferences. Scattered reports indicate significant latitudinal effects on $vitD_3$-synthesizing ability. Webb et al. (1988) reported ampule conversions of 3% in Los Angeles, California (34°N) and 10% for Puerto Rico (18°N), both in January. Gehrmann et al. (2004) report conversion at about 11% at noon in Boyd, Texas (32°N) in September. Conversion at Iquitos, Peru (3°S) was about 15% in February (Gehrmann, unpubl. data). Ampule photoproduct formation was characterized throughout the year in Edmonton, Canada (52°N) by Webb et al. (1988). Additional examples can be found in Chen (1999) and Holick (2004).

Knowing the UVB requirements for a species studied in captivity allows for more specific recommendations. For example, Ferguson et al. (2002) reported that lamps that produce conversions from 0.52% to 1.32% after a 2 h exposure when used for 12 h per day, facilitate the production of viable hatchlings in the panther chameleon. Conversion percentages above or below these values resulted in reduced hatchability of viable eggs. Referring to Table 3 and using the regression equations, we see that a blacklight with an irradiance of between 5 and 11 $\mu W/cm^2$, a Reptisun 5.0 between 15 and 33 $\mu W/cm^2$, an ESU Desert 7% between 20 and 42 $\mu W/cm^2$ and a Westron spot between 8 and 35 $\mu W/cm^2$ will produce conversions within the 0.52% to 1.32% range. It is suggested that further studies relating UVB irradiance to husbandry and reproduction in reptile species, especially lizards, will contribute to their captive welfare.

The percent of photoproduct formation in ampules can serve as a reference to doses and associated irradiances measured with other meters. For example, from Table 3 we see that for a Sylvania 350 blacklight the amount of photoproduct formed is 1.34% when the irradiance is 13 $\mu W/cm^2$. Using the data in Gehrmann et al. (2004) it can be calculated that 1.34% is associated with an irradiance of 7 $\mu W/cm^2$ measured with a Gigahertz-Optik meter, 80 $\mu W/cm^2$ measured with a UVX meter, and 17 $\mu W/cm^2$ measured with a Spectroline DM 300N meter.

The Solarmeter sensor and processor are combined as a single unit. Because the sensor is located about 10.5 cm above the meter bottom, it cannot be used to directly measure the irradiance at the substrate level in an enclosure. Nevertheless, this meter will be useful for many purposes, including monitoring the UVB output of various lamps or checking the attenuation of UVB by various materials.

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