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Medical cautery units as a permanent and non-invasive method of marking lizards

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Abstract. The identification of previously captured individuals is essential for a wide variety of ecological and behavioural studies. A lot of different methods are used for marking lizards, however they have many drawbacks. In presented study we used heat-branding method, using pen-like medical cautery units, previously employed to successfully mark other lizard species and snakes. The technique is permanent, readable and harmless for lizards, as well quick and easy. In 2009 we marked 111 individuals of sand lizard, *Lacerta agilis*. Next year we caught 88 lizards, 17 of them were re-captured. Among these re-captured lizards, five were caught after 26.8 (± 16.3) days (means in the same year) and 12 after 308.8 (± 64.3) days (means in the next year). Recaptured individuals were still unambiguously recognisable.

Keywords. Branding, sand lizard, less harmful method, resistant, *Lacerta agilis.*

INTRODUCTION

Many studies require individuals to be marked for identification. Accurate marking may be of great importance in ecological and behavioural studies, such as the determination of growth rates, movement patterns, reproductive histories, and other individual attributes that are critical to understanding species demography and population ecology (Fitch, 1987; Gaisler and Chytil, 2002; Venkatarama et al., 2008; Wanger et al., 2008; Angelini et al., 2010). Marking methods should be permanent, readable, as well as not harmful to the marked individuals (Murray and Fuller, 2000).

Lizards are usually marked by toe-clipping (Ikeuchi et al., 2005; Ferner, 2007). However, toe-clipping has drawbacks for reptiles and may negatively affect survival, mate acquisition, performance (Bustard, 1971; Blocha and Irschick, 2005; Schmidt and Schwar-

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zkopf, 2010) and endurance (Schmidt and Schwarzkopf, 2010). In addition, toe-clipping is painful and possibly stressful to the animals. Moreover, lizards sometimes naturally lose their toes (Hudson, 1996; Ribeiro and Sousa, 2006) which can cause incorrect identification of the animal. Toe-clipping, as well as other methods like clipping scales (Atzori, 2007), may induce infection (Weary, 1969). Sometimes microchips called passive integrated transponders (PIT tags) are used to uniquely mark lizards, but they are very expensive (Winne et al., 2006), not suitable for small vertebrates (Gibbons and Andrews, 2004) and may induce more stress than toe-clipping (Langkilde and Shine, 2006).

In some studies less harmful techniques are used, such as tagging lizards with bee marking kits or paint (Johnson, 2005). However, these methods are not permanent (Simon and Bissinger, 1983; Todd, 2005) and are only useful in short-term studies, mainly because lizards will lose such marks when they shed their skin or if they suffer skin abrasion against surfaces. Ribeiro and Sousa (2006) introduced lizard banding, using elastic hair fitted to the lizard's neck. This inexpensive method allows marking of lizards of small to moderate body size, causes no injury and does not appear to strongly influence lizard movements. However, animals have to have a head much larger than their neck diameter and coarse scales. Moreover, any conspicuous external markers may affect the lizard's traits, such as camouflage and hiding from predators. Such methods may also be unsuitable for studies of social status or mate choice if the focal species use colour for communication (Ribeiro and Sousa, 2006).

Alternatively, lizards can be individually marked by freeze-branding (Lewke and Stroud, 1974) or heat-branding (Ehmann, 2000). However, these methods traditionally have been difficult to apply in the field, because they required specialized equipment (e.g., liquid nitrogen) or an electrical current (Winne et al., 2006), and were considered time-consuming. In the current study we used and discuss of potential pro and cons of a heat-branding method, using medical cautery unit. It was previously successfully utilized in multi-year snakes research (*Seminatrix pygaea*, *Agkistrodon piscivorus*, *Coluber constrictor*, *Nerodia* spp., *Crotalus horridus*, *Cemophora coccinea*, *Lampropeltis triangulum*, *Tantilla coronata*; Winne et al., 2006). Snakes were individually marked, by branding 1-3 ventral scales anterior to the anal plate and extending the mark diagonally onto adjoining lateral scales. Medical cautery units were also successfully used for *Podarcis sicula* lizards marking (Vervust and Van Damme, 2009). In this case a first mark was cauterized on one of the first ventral scales on the right side of the lizard body, creating a reference point. Following marks were burned into other ventral scales and were given names reflecting their position with regard to the first mark. The lizards were kept for 48 hours after marking, and during that time researchers did not notice any obvious negative effects of the medical cautery unit on the animals.

MATERIALS AND METHODS

The study was carried out in April - September 2009 and April - June 2010. Sand lizards (*Lacerta agilis*) were caught one day in April, June and September 2009, three days in May 2009, one day in March, May and June 2010, and three days in April 2010. The study area were close to the Barycz valley in western Poland (51°34'N, 17°40'E, elevation 110-170m) and included gravel pits, meadows, forest edges and were surrounded with extensive used farmlands (for details see Ekner et al., 2008). Liz-

ards were captured using landing fishnets or by hand, then aged (adult, subadult and juvenile), sexed and measured (snout to vent length, SVL). We individually marked lizards by heat-branding them with "Aaron Medical Change-A-Tip cautery units" (http://www.boviemed.com). Medical cautery units are pen-like appliances with a hot wire at the end and are available in two temperature classes (1204 °C and 704 °C). We used the low-temperature class. We held a lizard in one hand and used the other hand to mark the lizard with the cautery unit (Fig. 1). The procedure took about 10 seconds.

We marked lizards with cauterized dots on the ventral surface of the lower jaw. We assigned appropriate digits to mental and submandibular scales, to form a unique numerical code (Fig. 2).

Fig. 1. Procedure of marking lizards using the medical cautery unit. Lizards are held in one hand and marks are left on mental and submandibular scales using the cautery unit held in the other hand.

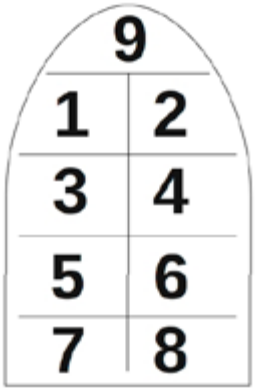

Fig. 2. A numerical code created on ventral part of the lizard's head. The code is created by dots, left on the particular scales using a medical cautery unit.

Fig. 3. Examples of the lizards marked using a medical cautery unit. We noticed each modification in the arrangement of scales. On the photograph specimens nr: "467/8" or "456/7 with double first scale".

We noted any aberrations in the arrangement of scales (Fig. 3). Lizard visible on the photo has only eight scales, hence, counting in sequence, it may have the number "467/8". However, it is only suggestion and because first two scales on the left side looks like one but double, the specimen could have a number "456/7 with double first scale". The details depend on the researcher, however the numbers should be assign consistently. Only numbers with ascending digits were used, e.g. so that 12 and 21 are not mixed up, because each combination of the digits is used only once in the marked population. Lizards normally have nine mental and submandibular scales, hence the method provides at least 511 possible number of combinations, in case of 1-9 of dots cauterized. The total number of possible codes that can be marked using two dots, three dots or four dots is 36, 84, 126, respectively. However, these are minimum numbers of combinations, because additional possibility is when considerate any aberrations in the arrangement of scales, mentioned above (Fig. 3).

Through the paper the results are shown as mean ± SD, or as a mean with 95% confidential limits, calculated by an Excel Macro for presence-absence data

RESULTS

During April-September 2009 we marked 111 *L. agilis* specimens. In March-June 2010 we caught 88 lizards in the area. At this time, 71 of the lizards were captured for the first time (80.7%, 95% CL: 70.9-88.3), and 17 were re-captured (19.3%, 95% CL: 1.7-29.1). Five of 17 re-captured specimens were caught once again in the same year after 26.8 (± 16.3) days from the first catch, others 12 were caught next year after 308.8 (± 64.3) days. All of the two groups of re-captured lizards were still unambiguously recognisable. However,

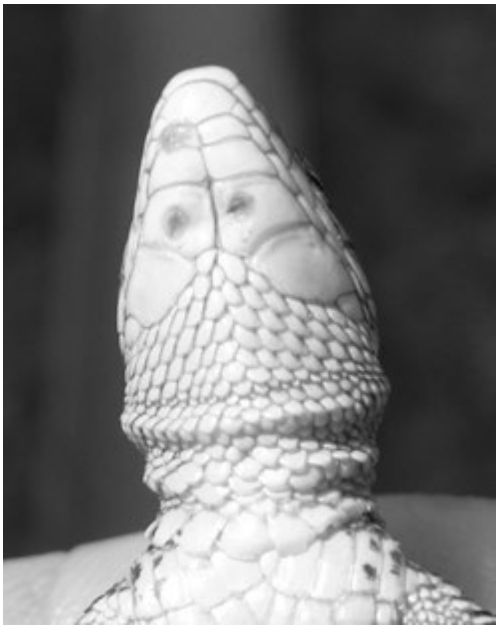

Fig. 4. The marks left on lizard scales re-captured after about one month (lizard no 156).

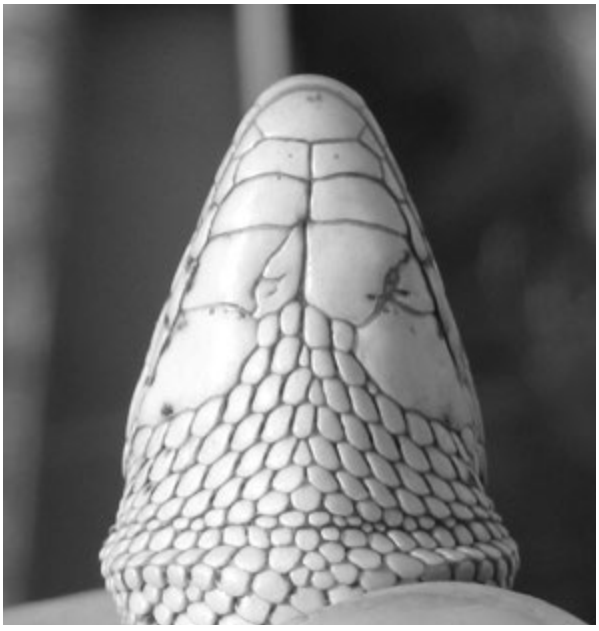

Fig. 5. The marks left on lizard scales re-captured after about one year (lizard no 56).

the marks left on lizard bodies re-caught after about one month (Fig. 4) were completely different from the marks left on lizards re-caught after about one year (Fig. 5). After one month marks on the scales are visible as a dots and are similar to those seeing immediately after the marking. Whereas, after a year marks look like cracks in the scales.

DISCUSSION

Almost 17% (12 of 71) of the marked lizards were re-captured after about one year. Such small number of re-caught animals can be caused by very big population living in a study area (more than 350). We successfully used a medical cautery unit to mark sand lizard *L. agilis*. After an average of one year marks were still well-defined. After examination, using a magnifying glass, we didn't notice any disappearance traits of the marks. The marks left on lizard bodies re-caught after about one year were different from the marks left on lizards re-caught after about one month and shortly after a branding. However, that marks were looking almost the same as those presented in other lizard's study (Vervust and Van Damme, 2009).

We didn't have an opportunity to re-visit the branded lizards later, however Vervust and Van Damme (2009) report that marks remained clearly visible over a period of at least 485 days during which the lizards resided in their natural habitat. Moreover, the marking procedure has proved to be effective for other reptiles (snakes) for more than three years (Winne et al., 2006). In that study marks were readable even as the snakes have tripled in length and increased many-fold in mass.

There are a lot of advantages of using this method. Medical cautery units are small, field-portable and inexpensive (about 30 USD, price in summer of 2010). This method is capable of being quickly and easily used in the field without any preliminary training and is not affected by the researcher skills. The short time (about 10 seconds) and the simplicity of the procedure likely make marking less stressful for lizards than many other methods. Research with less disturbance is especially important in studies investigating patterns of behaviour (Johnson, 2005). In our study animals didn't show any visible reaction to cauterizing, hence we think that this technique is relatively painless for lizards. Moreover, previous study showed the marking method does not have any obvious negative effects on reptiles (Winne et al., 2006; Vervust and Van Damme, 2009).

The method provides at least 511 possible number combinations, using up to nine marks per lizard. However, if study required to mark more lizards, dots could be cauterized on scales on other areas of the body, e.g. ventral scales, where even only four dots allow to marked as much as 197709 individuals (Vervust and Van Damme, 2009). It is a precise and easily visible way to identify particular individuals. If dots are left on the mental and submandibular scales or other ventral scales, marks are hardly visible for researches, but not conspicuous to predators or other individuals and hence probably do not affect survival or behaviour (Ribeiro and Sousa, 2006).

We recommended medical cautery units for lizard study, both in the wild and in laboratory conditions. We suggested to use the method for thick-skinned species, however to be honest we are not sure if it would be appropriate for reptiles with fragile and thin skin, like Gekkonidae, Phyllodactylidae or Sphaerodactylidae.

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