SALAMANDRA **53**(4) | 541–544 | 30 October 2017 | ISSN 0036–3375

Correspondence

Are Visual Implant Alpha tags adequate for individually marking European cave salamanders (genus *Hydromantes*)?

Enrico Lunghi[1,2,3] & Michael Veith[1]

[1)] Universität Trier Fachbereich VI Raum- und Umweltwissenschaften Biogeographie, Universitätsring 15, 54286 Trier, Germany
[2)] Museo di Storia Naturale dell'Università di Firenze, Sezione di Zoologia "La Specola", Via Romana 17, 50125 Firenze, Italy
[3)] Natural Oasis, Via di Galceti 141, 59100 Prato, Italy

Corresponding author: Enrico Lunghi, e-mail: enrico.arti@gmail.com

Manuscript received: 13 February 2017
Accepted: 9 May 2017 by Alexander Kupfer

During the last decades, studies on species ecology has improved considerably, becoming continuously more focused on individual needs (Emata & Marte 1992, Link & Hesed 2015, Mazerolle et al. 2007). To this end, researchers increasingly use Capture-Mark-Recapture (CMR) techniques, which allow to obtain ecological and behavioural data on individuals (Emata & Marte 1992, Pierce et al. 2014, Sanchez-Camara & Booth 2004). One of the crucial aspects is the selection of appropriate marking methods, a decision that may strongly affect the outcome of a study (Brannelly et al. 2014, Davis & Ovaska 2001, Robison-Cox 1998, Swanson et al. 2013). Therefore, many studies aim at evaluating the efficiency of marking methods (Buckmeier & Irwin 2000, Jerry et al. 2001, Lemarié et al. 2000, Potts 2012, Woods 2005) and related activities (Anholt et al. 1998, Kinkead et al. 2006) in various species.

Visual Implant Alpha tags (VIA; Northwest Marine Technology, Inc., Shaw Island, Washington) are one of the more widely used marking methods (Buckley et al. 1994, Heard et al. 2008, Measey et al. 2001, Osbourn et al. 2011). VIA are biocompatible tags (standard size 1.2 × 2.7 mm or large size 2 × 5 mm) that have black text on a fluorescent and coloured background on one side (see Fig. 1) meant for being implanted subcutaneously; their combination of an alpha-numeric code (one letter and two numbers) with four colours facilitates up to 10,000 different markings. Tags are inserted under the skin of animals by means of a specialized injector. However, despite the number of successful studies (Arce et al. 2003, Crook & White 1995, Emata & Marte 1992, Turek et al. 2014), this method seems to have some limitations that will prevent its use in various circumstances. Firstly, there may be handling problems related to tag implantation, e.g., the insertion of tags seems to be not that easy in some instances, requiring a team of experts and maybe controlled conditions, rendering this method not always practical in the field (Heard et al. 2008). In some species, anaesthesia and/or skin cuts are required to properly implant the tags (Buchan et al. 2005, Gower et al. 2006). Furthermore, tags may be accidentally flipped over during insertion or slip beneath muscle tissue, or come to rest under heavily pigmented skin, all of which will affect their readability (Heard et al. 2008, Isely et al. 2004, Wagner et al. 2013). Secondly, there are some concerns about tag retention, which seems to be not high enough in several circumstances (Isely & Grabowsky 2004, Pillai et al. 2009, Replinger & Wood 2007) and probably positively correlated with the sizes of individuals, making this method not the best choice to mark early life-stages of small-sized amphibian species (Davis et al. 2014, Isely et al. 2004). Finally, some authors reported that wounds produced by the injector may require a long time to heal, i.e., several days, consequently paving ways for tag expulsion and pathogen infections (Buckmeier & Irwin 2000, Heard et al. 2008, Replinger & Wood 2007, Robison-Cox 1998). As far as amphibians are concerned, VIA tags were tested only in few species (Buchan et al. 2005, Gower et al. 2006, Heard et al. 2008, Kaiser et al. 2009, Kinkead et al. 2006, Osbourn et al. 2011, Pittman et al. 2008). Considering the potential problems it is extremely important to test VIA tags in other study species prior to their application since the method may produce biased data.

Two types of marking techniques were successfully applied in previous studies on European cave salamanders (genus *Hydromantes*, see also Wake 2013), i.e., Visual Im-

© 2017 Deutsche Gesellschaft für Herpetologie und Terrarienkunde e.V. (DGHT), Mannheim, Germany
Available at http://www.salamandra-journal.com

plant Elastomers (Salvidio 2013) and photographic recognition (Salvidio et al. 1994); however, to the best of our knowledge, VIA was never tested before for them. Here we report on our tests and evaluation of VIA tagging in two species of European cave salamanders, *Hydromantes italicus* and *H. flavus*.

VIAs are provided as plastic sheets (100 tags each) in which the single tags are attached by at their short sides. To load the injector, a label must be placed inside the needle, and it is released from the sheet by twisting the injector. However, this sometimes produces an imperfect tag separation, creating sharp edges that represent a serious danger to individuals. In such cases we cut away the sharp parts using small scissors. Given the limited plasticity of tags (although being soft their shape will not change) and considering possible dangers due to sub-optimal tag loading, we preferred to apply tags only on limbs, as these body parts do not hold vital organs and can be regenerated if necessary (Lanza et al. 2006, Scaravelli et al. 2002). We also avoided tagging tails, as these salamanders are able to autotomise them (Lanza et al. 2006). We chose to apply standard-size VIAs only in individuals large enough to withstand the size of the needle (for standard tag ~2 mm). We first applied alpha tags on three individuals of *H. italicus* from a cave in the northern Tuscan Apennines (lat. 43.90°, long. 11.11°); however, the dark colouration of the ventral side obscured the tags even right after application, so that we aborted the test with this species. We then applied them to individuals of *H. flavus* from six caves in Monte Albo, northwestern Sardinia (lat. 40.53°, long. 9.63°). This species has a pale/white ventral colouration, so that tag readability would probably be better. Perforating the salamander skin with the injector was not always easy, and the large wound left behind by the injector needle allowed tags to easily slip out from their subcutaneous position. In these cases, we manually reinserted the tags using tweezers. In a few instances tags slid between muscles and became partially illegible, while in one case the tag flipped and became unreadable thus. Well-positioned tags were easily readable with bare eyes (Fig. 1). After tag application, individuals were observed for 15 minutes to monitor their physical well-being. Injections into *H. italicus* legs were performed by only one operator, while the application of VIA tag in *H. flavus* were carried out by at least two operators in most of the surveys. All operations were performed while wearing sterile latex gloves, and all equipment was disinfected with bleach and then washed with water after each survey.

We marked 114 individuals of *Hydromantes flavus* (61 females, 50 males, and 3 juveniles) with VIA tags. No salamander incurred any problematic injury. Time required for individual marking differed substantially depending on the number of operators, ranging from an average of 90 seconds needed by one operator to an average of 15 seconds with two operators. Within twelve surveys, we recaptured 14 different individuals (~12% of total marked), and only one of them was recaptured twice. None of these salamanders showed injuries. First recaptures were made after six days and last recaptures were made after 272 days. During our first revisits (within 14 days), four out of eight recaptured salamanders showed incompletely healed tagging wounds, making us aware of an apparent risk of tag loss. In fact, in two recaptured individuals, tags were partially hanging from the wound, and in one of them we had

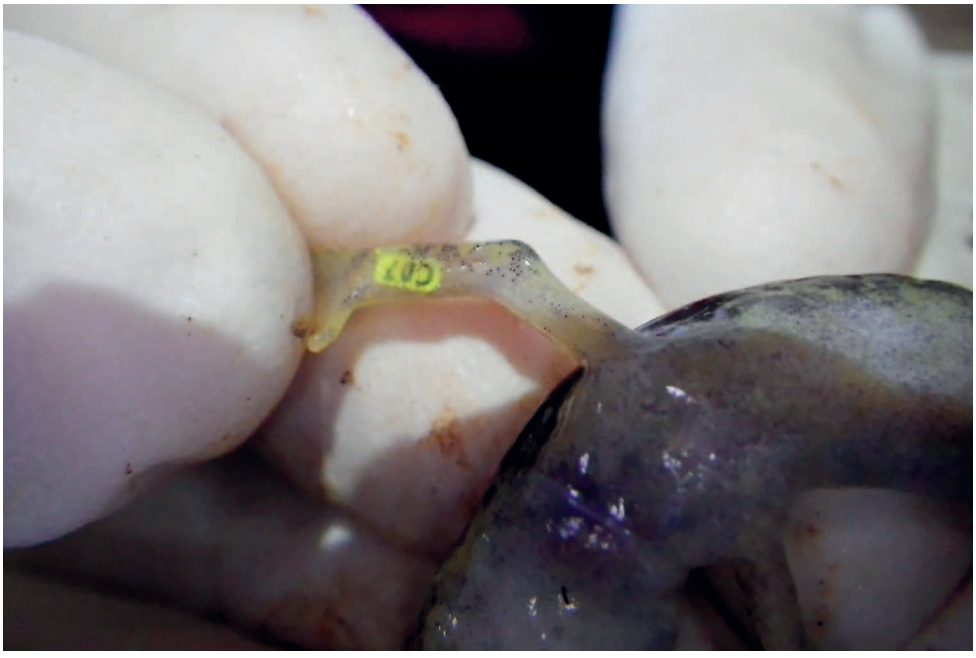

Figure 1. Example of a successfully implanted VIA tag in the forelimb of a *Hydromantes flavus*.

to implant a new one, as the previous tag was lost during handling. Proper tag retention (not counting flipped and slipped tags) in recaptured individuals occurred in 60% within the first 30 days and 50% thereafter.

Given the low number of recaptures, we were not able to provide any relevant information on the sizes of the studied populations. Thus, we only discuss the reliability of VIA marking technique here. VIA tags represent one of the newest commonly used marking methods, which seems to be fit for use in a wide range of species (Emata & Marte 1992, Gower et al. 2006, Turek et al. 2014, Woods 2005). Some of its advantages are the relatively low costs of tags (around 0.80–0.90 $ each) and a fixed code for each tag. However, the use of this marking method encounters several obstacles in some species, which may significantly influence the entire study outcome (Heard et al. 2008, Replinger & Wood 2007, Wagner et al. 2013). During our test of VIA tags on *Hydromantes*, we basically experienced all problems previously noted by other authors (Buckmeier & Irwin 2000, Davis et al. 2014, Heard et al. 2008, Isely & Grabowsky 2004). Our major concerns were related to the survival of individuals. The most risky phase of the marking procedure occurred during the insertion of tags, i.e., performing the injection without assistance was more or less impossible. Also, the needle size for standard tags basically equals the average width of limbs in adult *Hydromantes* and, considering both needle rigidity and length, it is easy to imagine that an imperfect application can be harmful to the salamanders. We did not use any anaesthesia, so the presence of a second operator was essential for keeping individuals immobile and succeeding in tag application. The size of the needle strongly limits the range of salamander species this method can be used on; thus, considering the body size of *Hydromantes* species (Lanza et al. 2006), only few adults and almost no juveniles were suitable for being marked with VIA tags. This renders this method unsuited for long-term studies aiming at recording data on, e.g., individual growth. Tag retention was low and probably influenced by the length of time wounds required to heal. Open wounds provided a chance for easily losing tags. This was observed in 50% of the individuals recaptured during the first 14 days, so that it is reasonable to assume that it may frequently occur in tagged salamanders. Seemingly low recapture rates may therefore plausibly explained simply by tag loss. Even if VIAs were retained by the marked salamanders, the readability of their tags was not always adequate since improper tag implantation may impede their detection.

Acknowledgements

We thank M. Mulargia for help in the field, S. Lötters for the review of an early draft, and A. Kupfer for commenting on the last manuscript versions. E. Lunghi was supported by The Mohamed bin Zayed Species Conservation Fund, Instrumentl and The National Speleological Society. Our study was conducted under Italian Ministerial Authorization 9384/PNM of 12/05/2015 issued by the Ministry of the Environment.

References

Anholt, B. R., S. Negovetic & C. Som (1998): Methods for anaesthetizing and marking larval anurans. – Herpetological Review, **29**: 153–154.

Arce, S. M., B. J. Argue, D. A. Thompson & S. M. Moss (2003): Evaluation of a fluorescent, alphanumeric tagging system for penaeid shrimp and its application in selective breeding programs. – Aquaculture, **228**: 267–278.

Brannelly, L. A., L. Berger & L. F. Skerratt (2014): Comparison of three widely used marking techniques for adult anuran species *Litoria verreauxii alpina*. – Herpetological Conservation and Biology, **9**: 428–435.

Buchan, A., L. Sun & R. S. Wagner (2005): Using alpha numeric fluorescent tags for individual identification of amphibians. – Herpetological Review, **36**: 43–44.

Buckley, R. M., J. E. West & D. C. Doty (1994): Internal microtag systems for marking juvenile reef fishes. – Bulletin of Marine Science, **55**: 848–857.

Buckmeier, D. L. & E. R. Irwin (2000): An evaluation of soft visual implant tag retention compared with anchor tag retention in channel catfish. – North American Journal of Fisheries Management, **20**: 296–298.

Crook, D. A. & R. W. G. White (1995): Evaluation of subcutaneously implanted visual implant tags and coded wire tags for marking and benign recovery in a small scaleless fish, *Galaxias truttaceus* (Pisces : Galaxiidae). – Marine and Freshwater Research, **46**: 943–946.

Davis, J. L., M. E. Barnes, J. L. Kientz & A. G. Galinat (2014): Effects of fish length and anatomical placement on retention of visible implant alpha tags in hatchery-reared rainbow trout. – North American Journal of Fisheries Management, **34**: 932–937.

Davis, T. M. & K. Ovaska (2001): Individual recognition of amphibians: Effects of toe clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. – Journal of Herpetology, **35**: 217–225.

Emata, A. C. & C. L. Marte (1992): The use of a visual implant tag to monitor the reproductive performance of individual milkfish *Chanos chanos* Forsskal. – Journal of Applied Ichthyology, **8**: 314–317.

Gower, D. J., O. V. Oommen & M. Wilkinson (2006): Marking amphibians with alpha numeric fluorescent tags: Caecilians lead the way. – Herpetological Review, **37**: 302.

Heard, G. W., M. P. Scroggie & B. Malone (2008): Visible implant alphanumeric tags as an alternative to toe-clipping for marking amphibians – a case study. – Wildlife Research, **35**: 747–759.

Isely, J. J. & T. B. Grabowsky (2004): Occurrence, size, and tag retention of sneaker male hatchery rainbow trout. – North American Journal of Aquaculture, **66**: 234–236.

Isely, J. J., D. G. Trested & T. B. Grabowsky (2004): Tag retention and survivorship of hatchery rainbow trout marked with large-format visible implant alphanumeric tags. – North American Journal of Aquaculture, **66**: 73–74.

Jerry, D. R., T. Stewart, I. W. Purvis & L. R. Piper (2001): Evaluation of visual implant elastomer and alphanumeric internal tags as a method to identify juveniles of the freshwater crayfish, *Cherax destructor*. – Aquaculture, **193**: 149–154.

Kaiser, K., M. Alloush, R. M. Jones, S. Marczak, K. Martineau & M. Oliva (2009): Use of visual implant alpha (vialpha) fluorescent tags in a small hylid frog with a new technique for application. – Herpetological Review, **40**: 421–422.

Kinkead, K. E., J. D. Lanham & R. R. Montanucci (2006): Comparison of anesthesia and marking techniques on stress and behavioral responses in two *Desmognathus* salamanders. – Journal of Herpetology, **40**: 323–328.

Lanza, B., C. Pastorelli, P. Laghi & R. Cimmaruta (2006): A review of systematics, taxonomy, genetics, biogeography and natural history of the genus *Speleomantes* Dubois, 1984 (Amphibia Caudata Plethodontidae). – Atti del Museo Civico di Storia Naturale di Trieste, **52**: 5–135.

Lemarié, D. P., D. R. Smith, R. F. Villella & D. A. Weller (2000): Evaluation of tag types and adhesives for marking freshwater mussels (Mollusca: Unionidae). – Journal of Shellfish Research, **19**: 247–250.

Link, W. A. & K. M. Hesed (2015): Individual heterogeneity in growth and age at sexual maturity: A gamma process analysis of capture–mark–recapture data. – Journal of Agricultural, Biological, and Environmental Statistics, **20**: 343.

Mazerolle, M. J., L. L. Bailey, W. L. Kendall, J. A. Royle, S. J. Converse & J. D. Nichols (2007): Making great leaps forward: Accounting for detectability in herpetological field studies. – Journal of Herpetology, **41**: 672–689.

Measey, G. J., D. J. Gower, O. V. Oommen & M. Wilkinson (2001): Permanent marking of a fossorial caecilian, *Gegeneophis ramaswamii* (Amphibia: Gymnophiona: Caeciliidae). – Journal of South Asian Natural History, **5**: 141–147.

Osbourn, M. S., D. J. Hocking, C. A. Conner, W. E. Peterman & R. D. Semlitsch (2011): Use of fluorescent visible implant alphanumeric tags to individually mark juvenile ambystomatid salamanders. – Herpetological Review, **42**: 43–47.

Pierce, B. A., K. D. Mcentire & A. A. E. Wall (2014): Population size, movement, and reproduction of the Georgetown salamander, *Eurycea naufragia*. – Herpetological Conservation and Biology, **9**: 137–145.

Pillai, B. R., L. Sahoo, K. D. Mahapatra, R. Ponzoni, S. Sahu, S. Mohanty, Vijaykumar & S. Sahu (2009): Evaluation of the new fluorescent internal tag (soft visible implant alphanumeric tag) in the freshwater prawn, *Macrobrachium rosenbergii*. – The Israeli Journal of Aquaculture, **61**: 345–350.

Pittman, S. E., A. L. Jendrek, S. J. Price & M. E. Dorcas (2008): Habitat selection and site fidelity of cope's gray treefrog (*Hyla chrysoscelis*) at the aquatic-terrestrial ecotone. – Journal of Herpetology, **42**: 378–385.

Potts, D. D. (2012): Investigating new methods to improve ageing and study movement patterns of larval great lakes sea lamprey (*Petromyzon marinus*) populations. – Department of Biology – University of Michigan, Flint.

Replinger, W. E. & J. B. Wood (2007): A preliminary investigation of the use of subcutaneous tagging in Caribbean reef squid *Sepioteuthis sepioidea* (Cephalopoda: Loliginidae). – Fisheries Research, **84**: 308–313.

Robison-Cox, J. F. (1998): A record linkage approach to imputation of missing data: Analyzing tag retention in a tag-recapture experiment. – Journal of Agricultural, Biological, and Environmental Statistics, **3**: 48–61.

Salvidio, S. (2013): Homing behaviour in *Speleomantes strinatii* (Amphibia Plethodontidae): A preliminary displacement experiment. – North-Western Journal of Zoology, **9**: 429–433.

Salvidio, S., A. Latters, M. Tavano, F. Melodia & M. V. Pastorino (1994): Ecology of a *Speleomantes ambrosii* population inhabiting an artificial tunnel. – Amphibia-Reptilia, **15**: 35–45.

Sanchez-Camara, J. & D. J. Booth (2004): Movement, home range and site fidelity of the weedy seadragon *Phyllopteryx taeniolatus* (Teleostei: Syngnathidae). – Environmental Biology of Fishes, **70**: 31–41.

Scaravelli, D., P. Laghi & C. Pastorelli (2002): Rinvenimento in natura di *Speleomantes italicus* (Dunn, 1923) con un arto rigenerato. – in: Salvidio, S. & M. V. Pastorino (eds): Primo convegno nazionale biologia dei geotritoni europei genere *Speleomantes*, Genova e Busalla (GE), 26 e 27 ottobre 2002, Programma e Riassunti, Università di Genova, Genova.

Swanson, J. E., L. L. Bailey, E. Muths & W. C. Funk (2013): Factors influencing survival and mark retention in postmetamorphic boreal chorus frogs. – Copeia, 2013(4): 670–675.

Turek, K. C., M. A. Pegg & K. L. Pope (2014): Short-term evaluation of visible implant alpha tags in juveniles of three fish species under laboratory conditions. – Journal of Fish Biology, **84**: 971–981.

Wagner, J. P., R. B. Blaylock & M. S. Peterson (2013): Evaluation of internal tag performance in hatchery-reared juvenile spotted seatrout. – North American Journal of Fisheries Management, **33**: 783–789.

Woods, C. M. C. (2005): Evaluation of vi-alpha and pit-tagging of the seahorse *Hippocampus abdominalis*. – Aquaculture International, **13**: 175–186.