2011. The Journal of Arachnology 39:194–196

SHORT COMMUNICATION

Suitability of a subcuticular permanent marking technique for scorpions

Kenneth J. Chapin: West Texas A&M University, Department of Life, Earth & Environmental Sciences, Box 60808, Canyon, Texas 79016 USA. E-mail: chapinkj@gmail.com

Abstract. The primary impediment of long term, high-resolution, ecological studies of scorpions is the difficulty of marking individuals for monitoring and recapture. I tested the use of Visible Implant Elastomer (VIE) as a permanent subcuticular tagging technique in the striped bark scorpion *Centruroides vittatus* (Say 1821). Mortality and prey capture rates of tagged scorpions did not significantly differ from untagged controls. Tag readability was high and comparable to published studies on other arthropod groups. Animals molted (3 treated, 7 control) and gave birth (1 treated, 2 control) successfully. I recommend VIE tagging as a viable solution to what was a major impediment to the proliferation of fine-scale ecological and population-level field research in *C. vittatus* and similar arthropods.

Keywords: *Centruroides vittatus*, mortality, prey capture, tagging, VIE

The primary impediment of long term, high-resolution ecological studies of scorpions is difficulty in marking individuals for monitoring and recapture. Scorpion tagging for ecological investigations has been restricted to various external paints (Sissom et al. 1990). Any external mark used with arthropods is lost with ecdysis. This limits researchers to two forms of long-term study: The first exclusively focuses on adults after their ultimate molt irrespective of immature individuals. This is impractical for species known to undergo postmaturation ecdysis and overlooks younger individuals. The second option is the inclusion of the highly inefficient and precarious practice of maintaining scorpion populations under near-constant observation to allow for the replacement of marks after ecdysis. Subcuticular tags injected just below the epidermal layer should remain within the animal during ecdysis and would therefore be permanent.

Visible Implant Elastomer (also termed Visual Fluorescent Injection Elastomer, or some variant of the two names; hereafter abbreviated as VIE) is a two part silicone-based animal tag injected hypodermically near the body surface as a liquid (Frisch & Hobbs 2006). The injection cures within the animal forming a pliable, biocompatible tag. The ability to read marks noninvasively by visual inspection is a prerequisite for many fine-scale field studies. VIE is highly pigmented in a variety of colors, allowing for visual identification of tags through transparent or semi-transparent material. Combinations of multiple tags in varying colors and injection sites allow for unique identifiers to distinguish tagged groups or individuals from one another. Additionally, commercial VIE is available in a variety of fluorescent colors — a seemingly appropriate attribute for scorpion marking, as ultraviolet light is perhaps the most common collection method for scorpion research.

Visible Implant Elastomer has been used extensively in fisheries management and has gained recent popularity in amphibian tagging. The use of VIE in arthropods has also gained popularity, but only among crustaceans including lobster, shrimp, crab, and crayfish (Claverie & Smith 2007; Pillai et al. 2007; Morgan et al. 2006; Buřič et al. 2008).

Few studies have measured the effects of tagging arthropods (only aquatic Crustacea represented) with VIE against untreated animals. The only report of increased mortality in treated animals compared with untreated controls was among 1.5-mo old *Homarus gammarus* Linnaeus 1758, but no significant difference was found within the same study among seven-month-old conspecifics (Linnane & Mercer 1998). Tag retention rate ranged from 82–100%, and readability ranged from 80–100%, though it should be noted that the dependence of these two measurements has not been addressed in any study reviewed. The most often noted concerns were errors in interpreting tag color (Curtis 2006) and, in a few cases, minor tag migration (Davis et al. 2004; Woods & James 2003) or fragmentation (Clark & Kershner 2006; Linnane & Mercer 1998). Two studies successfully injected particularly small specimens with mean weights (± SD) of 1.25 ± 0.5 g and 0.9 ± 0.8 g (Jerry et al. 2001; Pillai et al. 2007). These were also the only studies to show reductions in tag retention, though minor. Animals successfully molted while retaining tags during all studies reviewed.

No study of the use of VIE tagging with arachnids has been published. A different subcuticular mark, Passive Integrated Transponder (PIT) tags (a radio frequency identification technique) has been tested successfully in three large Theraphosidae species (Reichling & Tabaka 2001). Though the development of smaller (12.5 × 2.1 mm, 0.102 g) PIT tags in recent years has allowed for implantation of these devises in smaller animals, PIT tags can only physically fit in the largest arthropods. In addition to PIT tags, coded wire tags and visual implant alphanumeric tags were considered. Relative to the above tagging techniques, VIE is cost-effective with a minimally invasive application procedure, should impose minimal disruption to normal animal functioning, can be implemented on very small animals, and is not lost with ecdysis.

I here test the hypothesis that VIE tagging would not increase mortality or decrease prey capture in the terrestrial arthropod *Centruroides vittatus* (Say 1821).

METHODS

This study required a readily available scorpion species of moderate size. *C. vittatus* is locally abundant and is of moderate size, thereby increasing this study's range of inference for future field research. I included juvenile *C. vittatus* in the study to further demonstrate that VIE tagging can be used in small individuals and those that undergo ecdysis.

Colleagues and I collected *Centruroides vittatus* from Jeff Davis, Garza, and Randall Co., Texas, USA, on 26 September–22 November 2009. Each specimen was housed in a 16 oz (11.5 cm × 8 cm diam.) clear polyethylene container with a thin (ca 1 cm) layer of commercially purchased sand and a crumpled white paper towel to increase enclosure complexity and provide retreats. Small holes were put in the container's sides for ventilation. Containers were stored in an incubator averaging 28.3 ± 0.1° C SD and 30 ± 1.4% humidity.

Captive-bred house crickets (*Acheta domestica* (Linnaeus)) were offered to scorpions weekly and removed if not consumed after all other scorpions had fed (duration mean: 49 ± 13 min SD). The side of

each container was sprayed with tap water after prey capture to increase humidity and allow drinking from droplets.

I required collected scorpions to meet two criteria before being included in the study: Each individual had to survive in captivity for one month and capture prey within that time. I weighed animals meeting these criteria with an electronic scale (instrument error ± 0.01 g) and measured midline carapace length and mesosomal length with calipers (instrument error ± 0.2 mm). Scorpions included in the study had a mean ± SD weight of 0.34 ± 0.18 g, midline carapace length of 4.15 ± 0.69 mm, and mesosomal length of 13.84 ± 3.14 mm. The smallest animal weighed 0.07 g, had a 2.2 mm midline carapace length, and a 9.1 mm mesosoma length. Animals were randomly assigned to two equal groups. One group was randomly assigned for treatment by injection with VIE (n = 23; 8 males, 13 females, 2 juveniles) and the other acted as the study's untreated control (n = 23; 9 males, 10 females, 4 juveniles). I injected commercial red fluorescent VIE (Northwest Marine Technology™, Inc., Shaw Island, Washington, USA) dorsally through the posterior membrane of one of four randomly selected tergites using a 28 gauge, 0.3 cc syringe with a 13 mm beveled needle (BD™, Franklin Lakes, New Jersey, USA). This resulted in a longitudinal line of VIE positioned dorsolaterally just inferior to the cuticle. This location avoids the dorsal heart while maintaining tag readability. I followed a recommendation made by Godin et al. (1996) to position the tag parallel to muscle striation to avoid undue scarring and inflammation. I recorded the time (rounded to the nearest min) it took for each group to feed after injection.

I monitored treatment and control groups for 3 mo after tag implantation. I recorded if each individual captured prey during each feeding session. I also noted births, deaths, and ecdysis events. Volunteers inexperienced with reading VIE tags independently completed a test to determine tag readability (tag presence and placement) using ultraviolet light.

I totaled deaths in both groups at the study's end and performed a chi-square goodness of fit test to test for a difference in mortality between treated and control groups. I used Mann-Whitney U Rank Sum tests to determine if there was a significant difference in prey capture latency between treatment and control groups right after the tagging procedure, and over the entire study period. I conducted a Mann-Whitney U test concerning potential secondary variables that might have caused experimental error: mean animal weights, carapace lengths, and mesosomal lengths of each group. All statistics had an α value of 0.05.

RESULTS

Mortality of tagged individuals was not significantly greater than controls (10 and 9 individuals; $\chi^2_1 = 0.053$, $P = 0.818$). No treated animals died immediately after the injection procedure. Four control (17.4%) and five treated (21.7%) scorpions did not capture prey immediately after the injection procedure. Among scorpions that did feed, treated animals took a significantly longer time to capture prey (mean ± SD = 11.9 ± 26.7 min) than controls offered prey during the same feeding session (mean ± SD = 6.1 ± 8.1 min; $U_{19,18} = 94.50$, $P = 0.020$). The relative frequency of treated and control animals that captured prey during the feeding sessions was not significantly different ($U_{12} = 64.00$, $P = 0.664$). There was no significant difference in weight, carapace length, or mesosomal length between treated and control scorpions ($U_{23} = 195.00$, $P = 0.129$; $t_{44} = -2.002$, $P = 0.051$; $U_{23} = 188.50$, $P = 0.097$).

Two assistants observed twenty-three animals to test readability. Of 46 observations, only one resulted in a tagged animal identified as untagged (98% correct presence/absence observations). Three animals were identified with tags but incorrect tag placement, accounting for five misidentifications (89% correct placement observations) with both assistants misidentifying two of the same animals. During the study three treated and seven control animals molted and two tagged and one control animal gave birth. No patterns were found between these events and mortality or readability.

DISCUSSION

Survivorship of tagged animals did not significantly differ from the control group and was similar to those reported for other arthropods (Clark & Kershner 2006; Mazlum 2007; Claverie & Smith 2007; Pillai et al. 2007). Delay in prey capture among tagged animals was not surprising. It is reasonable to expect that animals handled and injected would exhibit delayed prey capture. Despite this result, mortality and feeding frequency did not differ between groups. While some short-term behavioral changes may result from the tagging procedure, this study found no evidence of any long-term impact of VIE injection. The three tagged animals that molted and one that gave birth did so successfully.

Tag readability was high, and within the 80–100% range indicated in studies of other arthropod groups. Assistants showed high consistency in tag identification. Both assistants made the same incorrect tag presence/absence determination, and two of the three same tag location misreads. This seems to indicate that the tagging procedure was to blame for misreads, and readability could near 100% with improved methods. Readability seemed to increase with experience in the VIE injection procedure. For this reason, I recommend practicing on preserved specimens and limiting the injection procedure to researchers with tagging experience.

Readability was not enhanced by the use of an ultraviolet light. Several commercially available VIE colors – including the red color used in this study – fluoresce brightly under ultraviolet light. When injected under scorpion cuticle, ultraviolet light induced the otherwise translucent cuticle to fluoresce, thereby obscuring the tag. Field researchers should read tags under white light, not ultraviolet. It should also be noted that VIE is not suitable for scorpion species with highly pigmented cuticle that will obscure tags.

I chose four dorsal mesosomal tagging locations because I postulated VIE in this area would impact the animals least. Tagging the metasomal segments or the trochanter, femur, or patella leg segments may result in slightly higher readability without increased mortality, but these locations have not yet been tested. More importantly, these alternate locations would increase the number of unique marks from 256 marks using four colors with the four locations tested in this study, to 5376 when also marking five metasomal segments – a number more than sufficient for long-term studies.

These results indicate that VIE is a suitable tagging alternative to traditional external marks in *Centruroides vittatus*. This study should encourage the proliferation of fine-scale ecological and population-level field research of terrestrial arthropods.

ACKNOWLEDGMENTS

I am grateful to Taylor G. Donaldson, Garrett B. Hughes, Richard T. Kazmaier, and W. David Sissom for thoughtful reviews of previous versions of this manuscript. Taylor G. Donaldson, Garrett B. Hughes, and Kari J. McWest helped collect scorpions. Special thanks to laboratory research assistants Jared Fuller and Daniel Nash for scorpion maintenance and participation in the readability test. Thanks to my graduate advisor, W. David Sissom, for his guidance and support. Funding was provided by West Texas A&M University and the Department of Life, Earth & Environmental Sciences. The Graduate School, the Department of Life, Earth & Environmental Sciences, and the Killgore Research Center of West Texas A&M University provided equipment and laboratory space.

LITERATURE CITED

Buřič, M., P. Kozák & P. Vích. 2008. Evaluation of different marking methods for spiny-cheek crayfish (*Orconectes limosus*). Knowledge and Management of Aquatic Ecosystems 389:1–8.

Clark, J.M. & M.W. Kershner. 2006. Size-dependent effects of visible implant elastomer marking on crayfish (*Orconectes obscurus*) growth, mortality, and tag retention. Crustaceana 79:275–284.

Claverie, T. & I.P. Smith. 2007. A comparison of the effect of three common tagging methods on the survival of the galatheid *Munida rugosa* (Fabricius, 1775). Fisheries Research 86:285–288.

Curtis, J.M.R. 2006. Visible implant elastomer color determination, tag visibility, and tag loss: potential sources of error for mark–recapture studies. North American Journal of Fisheries Management 26:327–337.

Davis, J.L.D., A.C. Young-Williams, A.H. Hines & O. Zmora. 2004. Comparing two types of internal tags in juvenile blue crabs. Fisheries Research 67:265–274.

Frisch, A.J. & J.A. Hobbs. 2006. Long-term retention of internal elastomer tags in a wild population of painted crayfish (*Panulirus versicolor* [Latreille]) on the Great Barrier Reef. Journal of Experimental Marine Biology and Ecology 339:104–110.

Godin, D.M., W.H. Carr, G. Hagino, F. Segura, J.N. Sweeney & L. Blankenship. 1996. Evaluation of a fluorescent elastomer internal tag in juvenile and adult shrimp *Penaeus vannamei*. Aquaculture 139:243–248.

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.

Linnane, A. & J.P. Mercer. 1998. A comparison of methods for tagging juvenile lobsters (*Homarus gammarus* L.) reared for stock enhancement. Aquaculture 163:195–202.

Mazlum, Y. 2007. Influence of visible implant fluorescent elastomer (VIE) tagging on growth, molting and survival of the eastern white river crayfish, *Procambarus acutus acutus* (Girard, 1852). Turkish Journal of Zoology 31:209–212.

Morgan, S.G., S.A. Spilseth, H.M. Page, A.J. Brooks & E.D. Grosholz. 2006. Spatial and temporal movement of the lined shore crab *Pachygrapsus crassipes* in salt marshes and its utility as an indicator of habitat condition. Marine Ecology Progress Series 314:271–281.

Pillai, B.R., S.C. Rath & S. Sahu. 2007. Evaluation of a visible implant fluorescent elastomer internal tag in juvenile freshwater prawn *Macrobrachium rosenbergii* (de Man). Indian Journal of Animal Sciences 77:1054–1056.

Reichling, S.B. & C. Tabaka. 2001. A technique for individually identifying tarantulas using passive integrated transponders. Journal of Arachnology 29:117–118.

Sissom, W.D., G.A. Polis & D.D. Watt. 1990. Field and laboratory methods. Pp. 445–461. *In* The Biology of Scorpions. (G.A. Polis, ed.). Stanford University Press, Stanford, California.

Woods, C.M.C. & P.J. James. 2003. Evaluation of visible implant fluorescent elastomer (VIE) as a tagging technique for spiny lobsters (*Jasus edwardsii*). Marine and Freshwater Research 54:853–858.

Manuscript received 14 October 2010, revised 2 February 2011.