

Short communication

A viable technique for tagging earthworms using visible implant elastomer

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Abstract

Earthworms perform many ecosystem services. However, due to their body shape, mucus covering, and subterranean behaviour, it has been very difficult to successfully tag individual animals for experimental purposes. This paper examines the potential use of commercially available visible implant elastomer (VIE) tags to mark earthworms. Two laboratory experiments were conducted employing four temperate earthworm species by injection of the VIE and making regular assessments. Results showed that the biologically compatible polymer tags, injected into earthworm muscle, remained in the animal and could be visually located after a period of 12 months. Tagging did not affect growth to maturity, mating or cocoon production of *Lumbricus terrestris*. VIE tagging can now be viewed as a viable technique for marking this and other earthworm species.

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1. Introduction

Many animal populations are relatively easily monitored because they are large, terrestrial, show diurnal activity and live in open habitat. Within such populations, individual animals may also be recognised from such attributes as specific colour patterns, scar tissue, hierarchical position or familial relationships. In addition, field observations can be enhanced by attaching appropriate tags to individuals e.g. wing tags or leg rings for birds, tattoos or ear tags on mammals or paint applied to the exoskeleton of certain insects (reviewed by [Southwood and Henderson, 2000](#)).

Nevertheless some groups of organisms, particularly soil dwelling invertebrates, have proved notoriously difficult to effectively tag, e.g. due to their mode of

locomotion, small size and/or subterranean behaviour. Some invertebrate groups have been successfully marked, such as large slugs, which have been tagged using a magnetic transponder ([Grimm, 1996](#)). However tagging earthworms remains difficult but is considered worthy of closer investigation because of the ecosystem services they provide ([Edwards and Bohlen, 1996](#)).

Tagging earthworms with a chemical marker has been tried with limited success. [Meinhardt \(1976\)](#) and [Mazaud and Bouché \(1980\)](#) used water-soluble coloured dyes to stain animals before releasing them into pasture soils to study population size, dispersal rate and mortality, but the dyes only persisted for a few months. Further workers, e.g. [Gerard \(1963\)](#), experimented with the use of radioactive isotopes, but these methods were highly invasive. More recently, [Dyckmans et al. \(2005\)](#) described a simple isotope tracer technique for earthworms, but this too is relatively non-persistent.

One innovative option for tagging earthworms was the use of commercially available visible implant

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elastomer (VIE) tags produced by Northwest Marine Technology (NMT) Inc., Washington, USA. These tags, already used successfully with fish and crustaceans (Godin et al., 1996; Brennan et al., 2005), are made from a medical grade material which is injected into the animal as a liquid but within 24 h cures into a soft, biologically compatible polymer. The VIE is supplied as two liquid components which are mixed and injected into the animal using a hypodermic needle. Tags ought to be visible to the naked eye if deposited in unpigmented tissue, but if in coloured tissue, fluorescent colours in the tag can be seen using a LED blue torch filtered with amber glasses. This paper seeks to establish through the use of VIE tags in two laboratory trials, a reliable, persistent, cost effective and non-toxic method for permanently marking earthworms.

2. Methods

Each trial made use of VIE tag material obtained from NMT, USA (see above). The colour selected for this work was a fluorescent yellow as this was in greatest contrast to tissues of most earthworm species investigated. Initially a trial pack of elastomer was utilised but this was supplemented with a “single color kit” which included all associated equipment consisting of: 6 ml of elastomer, a manual elastomer injector, mixing and injection supplies, a VI light, amber glasses, a VIE colour standard, a field carrying case and written and CD instructions.

In the two laboratory trials described, all earthworms were maintained in a temperature-controlled incubator (450 l series 3, LMS Ltd., Kent) at 15 °C and in constant darkness. The soil used was a Kettering loam and the food material was dried, rewetted horse manure. Lowe and Butt (2005) regard these as optimal

culture conditions for the temperate, soil dwelling species used.

2.1. Trial 1 (tagging four lumbricid species)

Earthworms used, at a range of developmental stages, were taken from stocks held in the laboratory, and consisted of *Allolobophora chlorotica* ($n = 5$), *Aporrectodea caliginosa* ($n = 2$), *Aporrectodea longa* ($n = 5$) and *Lumbricus terrestris* ($n = 9$). All of the latter were mature. The VIE was prepared following the written directions from NMT and was injected into the muscles of the earthworm body wall posterior to the clitellum region, to avoid body tissues associated with reproduction.

Each earthworm was washed in water, lightly dried with tissue paper and then held reasonably firmly by one researcher and injected directly from a syringe by a second researcher. Early attempts led to some injection into body cavities and some aborted injections where the needle passed completely through the earthworm. Nevertheless all animals received at least one and in some cases multiple VIE tags. Photographs were taken of each animal (Fig. 1a) and diagrams drawn of tag position(s) in each individual. A volume of 1 ml was prepared for this procedure and less than 0.3 ml was used for these 21 animals.

After tagging each earthworm was assigned a code and kept individually in a 600 ml plastic pot under conditions described above. Thereafter each pot was examined on a monthly basis (for 6 months) and then bi-monthly to 12 months. At each sampling, the presence/absence and position of any VIE tag was recorded, as was mass of each earthworm, reproductive condition and general health. As appropriate, soil and food material were replaced with fresh materials.

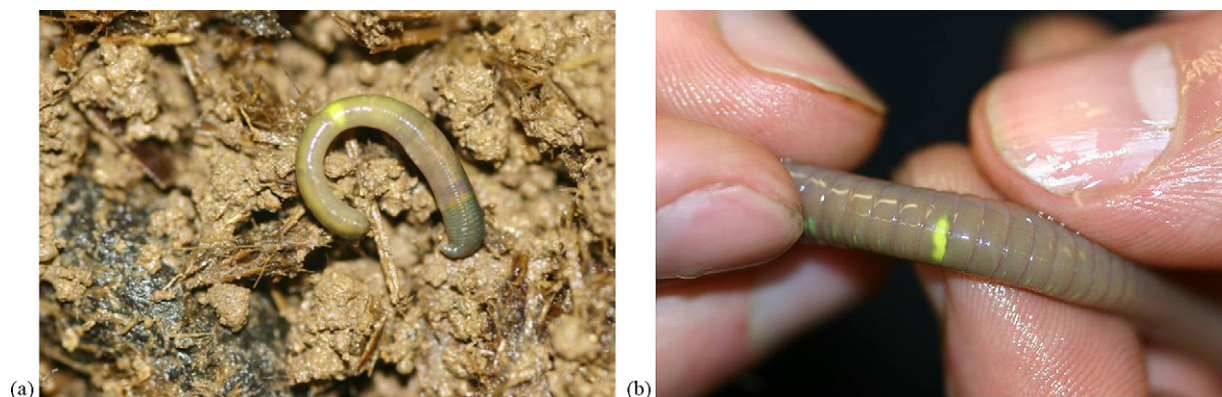


Fig. 1. Fluorescent yellow visible implant elastomer (VIE) tag in (a) *Allolobophora chlorotica* over three body segments; (b) *Lumbricus terrestris* within a single segment.

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