Use of visible implant elastomer and its effect on the survival of an endangered minute salamander

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Abstract
Use of visible implant elastomer and its effect on the survival of an endangered minute salamander. The population study of threatened species requires marking techniques that do not affect the survival of individuals. In this study, we evaluated the effectiveness of visible implant elastomer (VIE) in the identification and survival of individuals of the salamander *Parvimolge townsendi*. We compared three salamander groups under different treatments: intervened, simulated intervention and control. No significant mortality differences were observed between groups (with two, none, and one individual, respectively), but implant migration was observed in four of 10 intervened individuals. Although VIE does not have a significant effect on survival, implant migration should be considered before use in population studies.

Key words: Captivity, Marking techniques, *Parvimolge townsendi*, Survival, Threatened amphibians

Resumen
Utilización de implantes visibles de elastómero y sus efectos en la supervivencia de una salamandra enana en peligro de extinción. El estudio poblacional de especies amenazadas requiere técnicas de marcaje que no afecten a la supervivencia de los individuos. En este estudio, evaluamos la efectividad de los implantes visibles de elastómero (VIE, en sus siglas en inglés) en la identificación y supervivencia de individuos de la salamandra *Parvimolge townsendi*. Comparamos tres grupos de salamandras sometidos a diferentes tratamientos: Intervenido, Intervención Simulada y Testigo. No se observaron diferencias de mortalidad entre los grupos (con dos, ninguno y un individuo, respectivamente), pero se observó la migración del implante en cuatro de los 10 individuos intervenidos. Aunque los VIE no tienen un efecto significativo en la supervivencia, debería considerarse la migración de los implantes antes de emplearlos en estudios poblacionales.

Palabras clave: Cautiverio, Técnicas de marcaje, *Parvimolge townsendi*, Supervivencia, Anfibios amenazados

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Globally, more than 40 % of amphibian species are considered threatened (IPBES, 2019). Population and community studies of these vertebrates are essential to define their current situation and help in decision–making in response to the global crisis. Alternatives for studying amphibians in the field include mark and recapture methods, the effectiveness of which depends on the natural history of the species of interest, the resources available (including time and funding), and even the expected number of individuals to mark (Heyer et al., 1994). In addition, the method used must comply with the assumptions of generating unique, easy–to–interpret and persistent marks with the minimal impact on survival or detection (Campbell–Grant, 2008).

Numerous marking techniques in amphibians have been developed over the last century, such as surface markings, hypodermic staining using paints or labels, radio transmitters, passive microchips (PIT) and amputation of phalanges. Although this latter technique is one of the most commonly used marking approaches in this group, it is the most invasive approach (Heyer et al., 1994). Furthermore, it can significantly affect survival, growth, reproduction, locomotion and recapture probability (Perry et al., 2011). Its use is therefore controversial, especially in rare or threatened species (Phillips and Fries, 2009).

One marking technique that has enabled population studies of amphibians, even in those with a different natural history, is the use of visible implant elastomer (VIE) tags, developed by Northwest Marine Technology Inc. This technique consists of a fluorescent biocompatible polymer that is externally visible after being injected into areas of transparent tissue. The success of VIE has been reported in the marking of small amphibians (< 50 mm of snout–vent length, SVL; Marold, 2001) and in threatened species (Bendik et al., 2013). Although only minor problems have been documented concerning the physical capacity (Kinkead et al., 2006; Hoffmann et al., 2008) and survival of individuals (Campbell–Grant, 2008), migration of marks to other regions of the body (Campbell–Grant, 2008; Phillips and Fries, 2009) and even partial or complete rejection of the implant (Hoffmann et al., 2008) have been reported. Defining the effectiveness of this technique is therefore a necessary step to achieve reliable population or community studies that do not significantly affect the survival of individuals.

Parvimolge townsendi (Dunn, 1922) is a salamander of the family Plethodontidae. It measures a maximum of 60 mm in total length (TL) (fig. 1A). An endemic species to Mexico, it inhabits the Sierra Madre Oriental, between 800 and 1,800 m.a.s.l. and is mainly found in fragments of tropical montane cloud forest and oak forest (mostly species of genre *Quercus*) (Parra–Olea et al., 2008). Distribution of this minute salamander has recently been reduced (≈ 1,605 km^2) due to habitat loss resulting from the expansion of livestock and agricultural activities (Sandoval–Comte et al., 2012). This species is listed as Critically Endangered by the IUCN (Parra–Olea et al., 2008), while the Mexican government considers it in the category of Threatened (Amenazada) in the NOM–059–SEMARNAT–2010 (SEMARNAT, 2015). It is therefore necessary to identify techniques that allow us to monitor this species, minimizing the risk of mortality of the individuals studied. For this reason, we aimed to evaluate the impact of the use of VIE tags on the survival of *P. townsendi*, and to determine its effectiveness as a marking method for identifying individuals.

In October 2015 in the Área Natural Protegida Santuario del Bosque de Niebla Francisco Javier Clavijero (19.51014 ºN; 96.94332 ºW) in Xalapa, Veracruz, Mexico, we manually collected 30 adult individuals (18 females and 12 males) of *P. townsendi* through visual encounters (due to their threatened status). Total length of individuals ranged between 36 and 51 mm. In the first 60 minutes after capture, individuals were transported to the laboratory where they were randomly selected to receive one of three treatments (N = 10 individuals per treatment): a) intervened, consisting of the subcutaneous application of two red implants using insulin syringes of 30 units (0.25 x 8 mm); b) simulated intervention, the same treatment as for group a, but without applying the VIE; and c) controls, with handling only at the time of capture and no intervention. The punctures were performed without anesthesia, in the ventral area near the anterior and posterior members as well in the tail base; considering the bilaterality, six possible regions were considered according to MacNeil et al. (2011) (fig. 1B, 1C). Once the treatment was applied, the individuals were held captive for observation for 31 days.

While in captivity, the salamanders were placed individually in 500 ml transparent plastic containers, with approximately 80 % of the space occupied by leaf litter.Temperature and air humidity averaged 20.7 ºC (SD = 1.0 ºC), and 76 % (SD = 4.0 %) respectively. Survival of individuals was confirmed every day. Leaf litter was humidified daily and replaced every week. The new leaf litter in the containers included diminutive invertebrates that could act as potential prey for salamanders. After 31 days, all the surviving individuals were released at the site where they were captured.

During the experiment, no immediate effect of VIE was observed in individuals; all were active after the different treatments were applied. Three of the 30 individuals included in the experiment did not survive; two from the Intervened group and one from the control group. The first was a male (SVL = 22.5 mm) from the intervened group who died 14 days after capture and marking. The other two died at 25 days after capture, a female (SVL = 20.7 mm) from the Intervened group and a male (SVL = 22.3 mm) from the Control group.

To assess survival differences between treatments, we developed a contingency table with the proportion of live and dead individuals at the end of the study. Between treatments, the survival of individuals did not differ significantly (χ^2 = 2.22, df = 2, P = 0.32). Low mortality with this marking technique has already been reported for other salamander species such as *Desmognathus monticula* (with size larger than 50 mm of SVL) and *Desmognathus fuscus* (SVL < 50 mm;

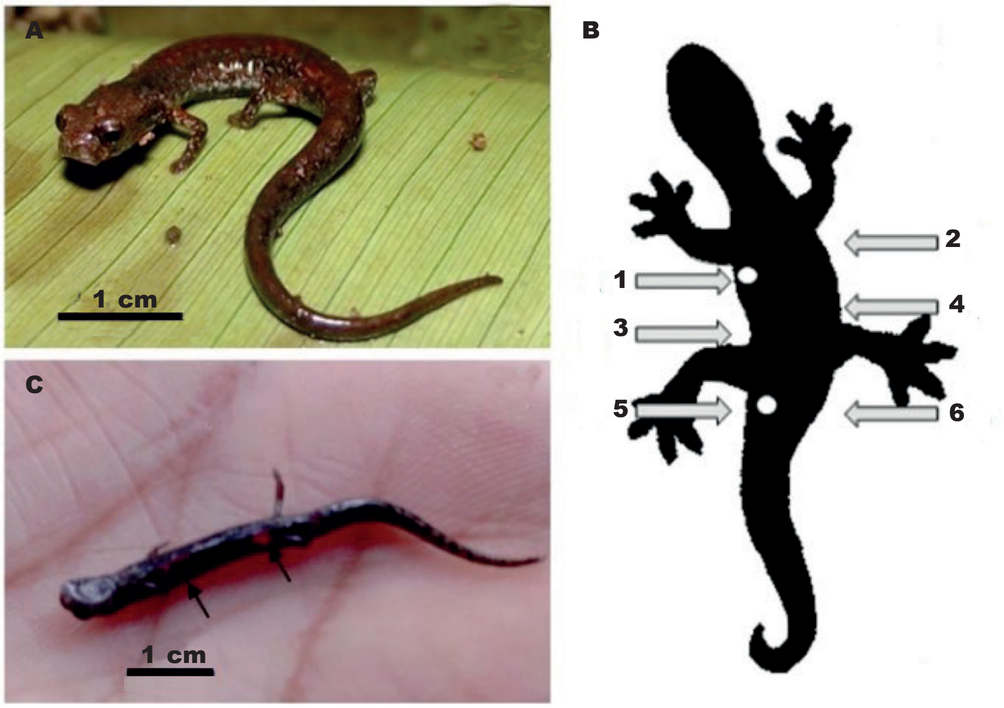

Fig. 1. *Palvimolge townsendi*: A, without visible implant elastomer, VIE; B, representation of six possible regions for the visible implant elastomer placement in salamanders,white dots denote the location of VIE in regions one and five from ventral view; C, immediately after VIE application in regions one and three.

Fig. 1. Palvimolge townsendi*: A, sin implante visible de elastómero, VIE; B, representación de seis posibles regiones en las que se pueden colocar el VIE en salamandras, los puntos blancos representan la ubicación de los VIE en las regiones uno y cinco desde una vista ventral; C, inmediatamente después de aplicar los VIE en las regiones uno y tres.*

Kinkead et al., 2006), for whom mortality events after two weeks of intervention were not reported. Likewise, the case of *Eurycea nana*, a tiny salamander (SVL < 40 mm), presented a mortality of marked individuals from 3 % at 30 days in captivity, to 17 % at the 244th day, while the mortality of the Control group ranged from 11 % by the 30th day to 22 % by the 244th day. The authors concluded that VIE does not have has not important effect on survival according to their studied animals (Phillips and Fries, 2009).

The salamanders in this study did not expel implants, but four elastomers migrated in four individuals: this migration occurred in three implants in an anteroposterior direction, while one implant migrated sideways (left to right from ventral view). The change of implant position in the body of animals may lead to misidentification of individuals and generate unreliable estimates of populations (Yoshizaki et al., 2009). To minimize this error, the marking of limbs of larger species (> 50 mm SVL) is recommended because in minute species such as that used in this study, limbs are almost as thin as the syringe used (e.g. genus *Thorius*). We found that VIE did not significantly affec survival of *P. townsendi* compared to the simple handling of specimens. However, because implants can migrate in the body of individuals of species such as that studied here, their use for individual identification should be conducted with caution. It is recommended to carry out pilot studies (before fieldwork) with the species of interest to evaluate the effectiveness of the marking technique used in this study. The use of complementary techniques such as marks with different color codes or the use of photomarking could avoid or reduce misidentification of individuals.

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