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Evaluating retention of Subcutaneous Passive Integrated Transponder (PIT Tag) to individually identify captive Lake Pátzcuaro salamanders *Ambystoma dumerilii*

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Abstract

Ambystoma dumerilii is a Critically Endangered amphibian endemic to a single lake in Mexico. The species is the focus of conservation efforts which include individuals maintained in captivity for captive breeding. To manage captive colonies effectively, and to monitor the wild population, a means of individual identification is required. This study marked N=80 captive individuals subcutaneously with a passive integrated transponder (PIT) tag to monitor the rate of retention. Body weight and behavioural observations were used to monitor the impact on the individual. Tag retention was 97.5 % across the population with no observable health impacts noted.

Background

The Lake Pátzcuaro salamander, *Ambystoma dumerilii*, is a Critically Endangered species of neotenic fully aquatic ambystomatid salamander, endemic to the Michoacán state of central Mexico (IUCN SSC Amphibian Specialist Group 2020). It has a restricted distribution of a single lake, Lake Pátzcuaro, the third largest lake within Mexico occurring at 2035 masl (Torres 1993). The lake is considered as potentially one of the most well studied and important lakes in Mexico (Torres 1993; Medina-Orozco et al. 2019) as it harbours several endemic taxa including fishes of the genus Chirostoma, *Algansea lacustris, Allotoca diazi*, a crayfish *Cambarellus patzcuarensis*, and the salamander *A. dumerilii*.

Ambystoma dumerilii has important cultural, economic, alimentary and perceived medicinal significance within the local community of Pátzcuaro (Velarde-Mendoza 2012) and was heavily exploited, but the fishery yield reduced from 6,242 kilos in 1987 to almost zero in 1999 (Huacuz-Elias 2002), and the species is now considered close to extinction in the wild (IUCN SSC Amphibian Specialist Group 2020), following a global trend in amphibian declines (Pounds et al. 2006; Alford et al. 2007; Wake and Vredenburg 2008; Luedtke et al. 2023). The decline of this species in the wild has been attributed to degradation of the quality of Lake Pátzcuaro, mainly linked to nutrient enrichment and eutrophication originated by wastewater effluents, fertilisers for agriculture and intensive farming, soil erosion and runoff from deforested uplands, reduction of the surface and depth of the lake, diffuse pollution and water quality deterioration of the lake (Torres 1993; Bravo-Inclán et al. 2022). Also historic over exploitation, introduced predatory fish species and parasites have contributed to the decline of *A. dumerilii* (Huacuz-Elias 2002; Poly 2003; IUCN SSC Amphibian Specialist Group 2020).

Although, previous to, and following this believed large-scale population decline, the species remains poorly understood within the lake (Brandon 1970a); much of all that is known was published by Huacuz-Elias (2002) for the natural population and the species reproductive biology, whereas behaviour, breeding and rearing in captivity was described by Brandon (1970a; 1970b; 1972; 1976; 1977), Huacuz-Elias, (1999), Saldaña et al. (2006) and Bland et al. (2021).

This species is maintained live in captive collections with colonies currently held within multiple institutions in Mexico, and within European Association of Zoos and Aquariums collections (EAZA) in Europe as an ex-situ program species (EEP). The primary aim of these captive colonies is to facilitate the conservation and appropriate zoo based research of this species; they may also represent a genetic resource to ensure that the optimum genetic diversity of this species is maintained in captivity on a long-term basis, with the ultimate aim of future reintroductions if necessary in order to establish, supplement or maintain a viable wild population (Bland et al. 2021). To effectively manage the captive populations in terms of reproductive individuals, individuals of specific genetic importance, monitor an individual's health and medical history, and to facilitate the study of the wild population, the recognition of individuals or groups of individuals is essential (Heyer et al. 1994). However, this species lacks individual natural markings and features making recognition of individuals impossible when maintained in large numbers, this is problematic when developing captive breeding management programs and when conducting studies of the remaining wild population. Furthermore, due to the regenerative capabilities of this species and welfare concerns aligned with modern practice, the use of traditional methods of marking such as toe clipping are rendered ineffective; clipped digits and other tissue will be regenerated (Bland et al. 2021). Therefore, suitable methods of marking individuals of this species need evaluation and development. Although methods of marking amphibian species have previously been developed using multiple genera, orders and varying techniques (Bailey 2004; Grant 2008; Whiteman et al. 2016; Le Chevalier et al. 2017; Tapley et al. 2019), due to the individual characteristics frequently observed in the biology of amphibian species, a suitable marking technique may be specific to a species, and the viability of an appropriate method may require individual species specific validation to determine the suitability and effectiveness of use. Owing to the slightly invasive nature of PIT tagging it is also important to evaluate potential health impacts this may have on a species, particularly for use in conservation programs or for animals tagged in the field and immediately released (Le Chevalier et al. 2017), such as fungal skin issues due to disruption of skin microbiome following tagging (Antwis et al. 2014).

This study aimed to investigate the use of subcutaneous passive integrated transponder (PIT tag) to mark individual *A. dumerilii*, a method successfully used to permanently and individually mark amphibian species (Ryan et al. 2014, Whiteman et al. 2016, Le Chevalier et al. 2017; Weber et al. 2019), in order to validate this method of identifying subadult and adult individuals of *A. dumerilii*. This study aimed to optimise the management of captive colonies for conservation breeding purposes, and to determine the suitability of this technique as a method of identifying individual animals to facilitate mark recapture studies of the wild population enabling ecological and population studies within Lake Pátzcuaro.

Action

During April 2018, sub adult and adult individuals (N=80) of *A. dumerilii* were PIT tagged subcutaneously using Trovan ID-162B/1.4 Animal Implant Mini Transponders using a Trovan mini transponder applicator in the right flank within the following collections; Chester Zoo (CZ N=13), Centro Regional de Investigaciones Pesqueras (CP N=26), Universidad Michoacana de San Nicolás de Hidalgo (UN N=13) and the Monastery of the Dominican Order in Pátzcuaro (MN N=28). Following marking a sample (N=51, Collections: CP, MN and UN) of this population had the body weight (g) recorded and the resulting data were used to monitor individuals following marking; body weight was used as the primary indicator of health in adult specimens following implantation of PIT tag. Individual PIT tags weighed 0.027 g and had a dimension of 14 mm x 8 mm, the implanter gauge was 2.12 mm. The size classes chosen for PIT tagging were selected due to the larger size of the individuals.

Weight data were collected from N=51 individuals upon tagging, then initially 20 days following implanting of PIT tag, and subsequently monthly. All N=80 individuals were reported on success of retaining the tag, and migration of tag to other areas of the body. During a >175 day period mortality of specimens was recorded and where possible, a postmortem examination was used to identify cause of death. Data was tested for a significant loss in body weight twenty days following handling and PIT tag implantation, as a rapid drop in body weight shortly following implantation of the PIT tag was considered an indicator of the effect of the handling and tagging on the salamander; body weight, general visual health condition, including signs of infection or fungal skin growth at the implant site, of the specimens and observations of abnormal behaviour such as spontaneous metamorphosis, and changes such as restlessness and increases of activity indicative of stress following PIT tagging were also recorded during daily husbandry.

Methodology for PIT tagging was as follows; Tagging required two persons both using powder free nitrile gloves, the first removed the specimen from water where it was gently restrained in the hands using a wet cloth to prevent uncontrolled movement during tagging, leaving only the required area of body exposed. The second person then undertook the implanting of tag using a Trovan PIT tag applicator, implanting in the lower right flank approximately 3-5 costal grooves upward of the lower limbs, dorsally to ventrally within a costal fold (Figure 1). The tag was then maintained in place by gravity when the salamander was returned to aquaria, minimising migration risk of the tag upwards and towards the entry site, tissue adhesive was not necessary to close the entry site, which closed and healed with no after care required. After tagging the salamander was placed into a temporary container of water for a short period of observation of up to 15 minutes, specimen was then returned to aquaria and maintained under regular husbandry practices as described by Saldaña et al. (2006) and Bland et al. (2021). Individuals were then inspected for retention using a LID-560ISO Pocket Reader (©Trovan) during handling for morphometric data collection as previously detailed.

Consequences

Tag retention and impact

The sample population in which body weight data was collected (N=51) began with a mean snout vent length of 13.80 cm ± 1.52 cm and weight of 120.91 g ± 44.27g prior to implantation of PIT tag; the smallest individual marked measured 10.50 cm SVL and weighed 54.90 g and the largest 17.0 cm SVL and 226.71g. Twenty days following handling and PIT tagging a paired repeat measure t-test showed no significant loss in body weight was observed ($t=-1.962$, $df=50$, $P=0.055$) with an increasing trend of weight gain, and no mortality of individuals was recorded. Body weight was observed to maintain and over a four-month period a 12 % increase of average body weight was noted across the sampled population (mean weight August 2018 135.46g ± 44.07g) (Figure 2). Although, 19 of the N=51 individuals lost a small amount of body weight following twenty days post tagging, with the greatest loss being 16 % of body weight in one individual, each of these then continued to gain and maintain weight for the remainder of the study.

In the >175 days following tagging an observed loss of two PIT tags from adult salamanders occurred across the entirety of the tagged population (N=80), in one instance the tag was found within

aquaria and was presumed to be rejected through the skin of the salamander, although no apparent exit sites on the dorsum could be distinguished. Throughout the duration of the study there were three mortalities within three separate colonies (CZ N=1, MN N=1, UN N=1), visual or behavioural health issues were not observed preceding death of the individuals, a post mortem report from one specimen (CZ) found abnormalities of the liver as a primary and likely cause of death, post mortems were not available for the further two individuals. Retention rate of subcutaneous PIT tag was 97.5 % throughout the entirety of the PIT tagged population (N=80) over a > 175 days period, with no observed adverse health effects, no recorded tag migration, spontaneous metamorphosis or abnormal behaviour. The high level of retention in this instance (97.5 %) is comparable to successful retention of PIT tagging observed in other Ambystoma species; 100 % N=9 *Ambystoma annulatum* (Ousterhout and Semlitsch 2014) and 100 % N=20 *Ambystoma tigrinum nebulosum* (Whiteman et al. 2016).

Weight was used as the indicator of health and stress in adult individuals as this is the predominant fluctuating factor of an adult specimen, and a significant loss of weight is a more common sign of ill health and stress in captive amphibians (Wright and Whitaker 2001), as is abnormal behaviour such as spontaneous metamorphosis in neotenic salamanders (Brandon 1976; Voss et al. 2009). Monthly intervals of collecting weights from specimens was decided upon as it was considered that excessive handling of this species may be a cause of stress and therefore negatively affect the health of the animal. The mortality of three individuals did not appear to be associated with the implantation of a PIT tag and all occurred greater than three months following tagging with no previous health effects observed. The liver abnormality observed in the single specimen (CZ) on postmortem is not considered to be an effect associated with PIT tagging, as an extensive review by Ferver and Moriarty (2007) of marking techniques of amphibians and reptiles shows no evidence of PIT tags resulting in liver damage in amphibians.

PIT tagging method

The methodology described here of the subcutaneous PIT tagging in Ambystoma differs from a methodology previously described used to PIT tag by Whiteman et al. (2016) for the comparable in size species *Ambystoma tigrinum nebulosum*. Whiteman et al. (2016) first used a scalpel to make an incision before using a needle to create a path through the epidermis for the tag to pass through, then manually inserted the tag underneath the dorsum with a finger. Highlighted by the authors in the description of this methodology, the tag may on occasion require manipulating several times to find the correct entry site created by the needle. The benefits of the methodology described in this report includes the ease of use of the Trovan mini transponder applicator as this ensures a very short period of restraint and implant time, which is performed in a single action and requires no further manipulation. The use of tissue adhesive was not necessary as has been used

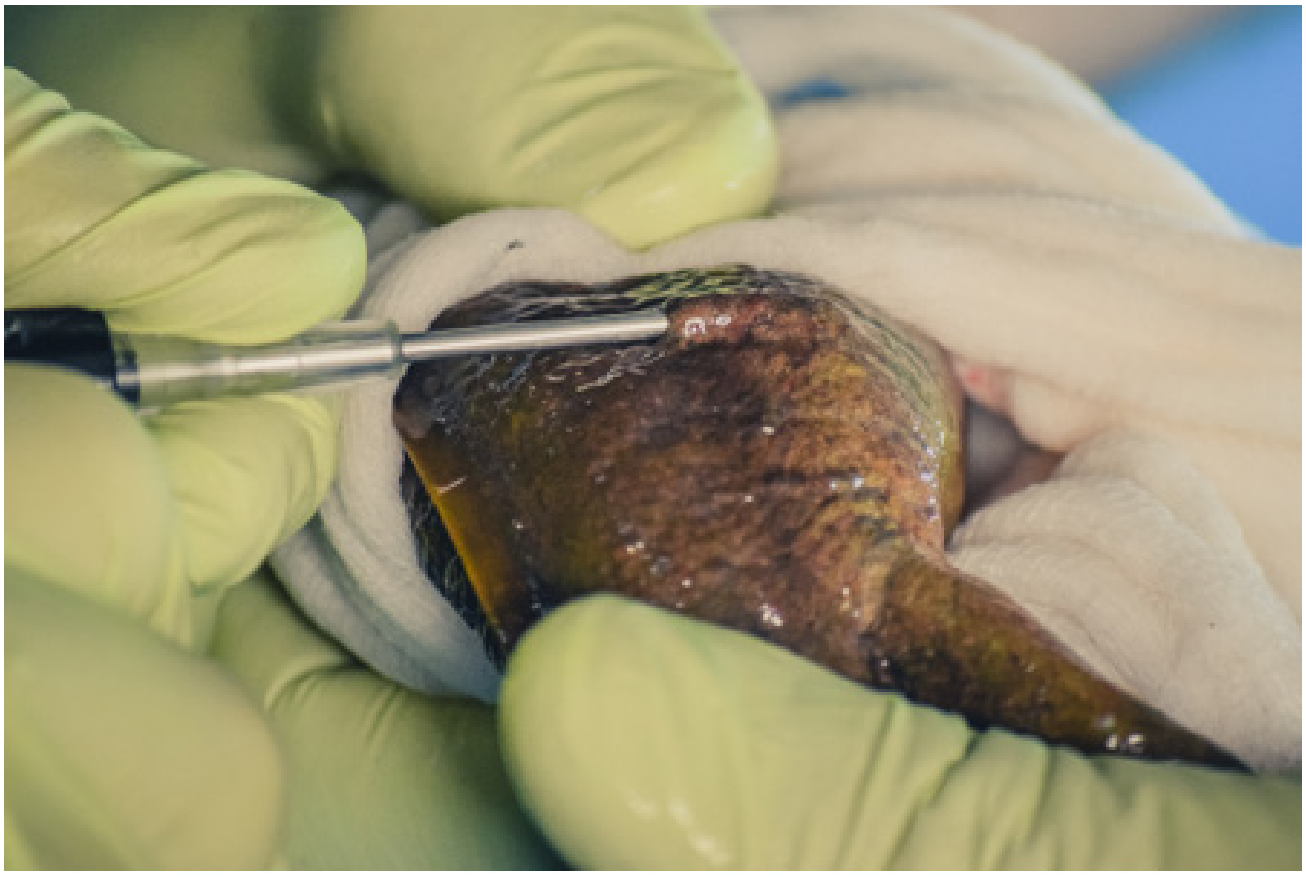

Figure 1. Methodology for PIT tagging adult specimen of *Ambystoma dumerilii*, specimen is gently restrained as PIT tag is implanted subcutaneously dorsally to ventrally within a costal fold.

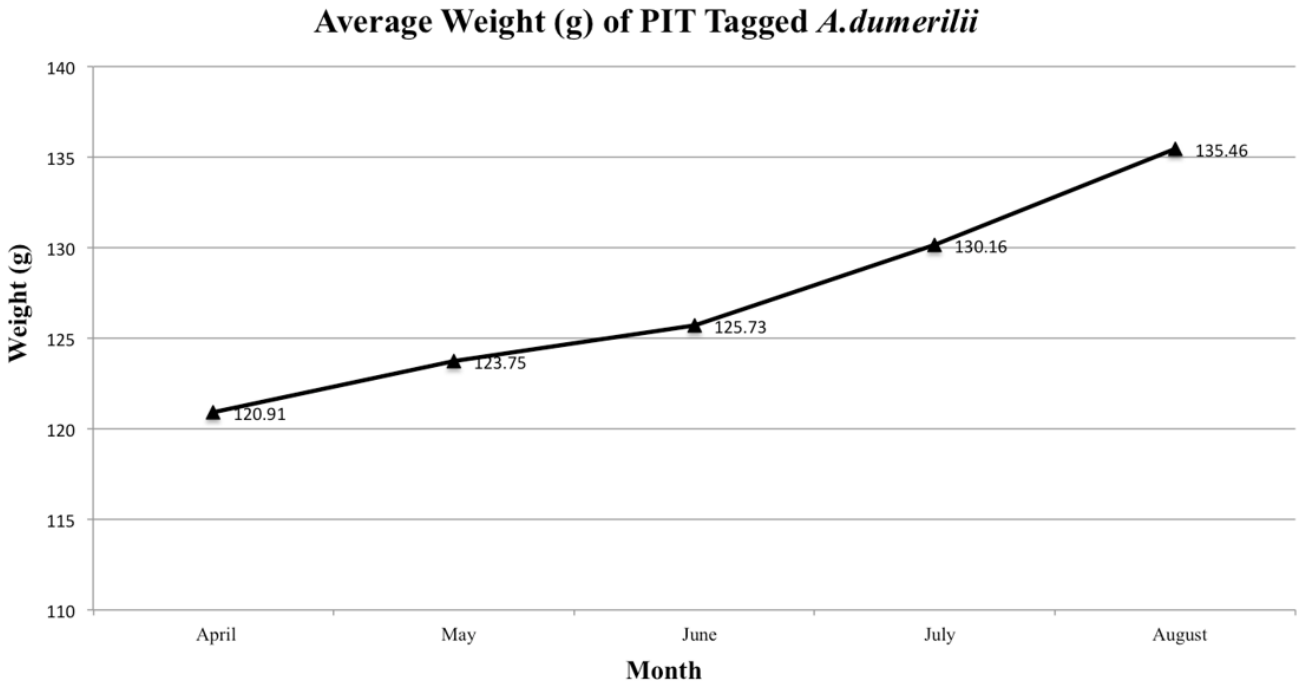

Figure 2. Average weight of sampled population of *A. dumerilii* following PIT tagging.

when tagging other species of Ambystoma (Ott and Scott 1999), and its use may have increased the total time of restraint and potentially added a further complication to the methodology. In a captive setting tagged individuals can then usually be easily identified without further restraint, as the PIT tag is readable through standard aquarium glass (Figure 3).

The period of tagging and data collection took place outside of the breeding season, where the increase of average body weight

Figure 3. Adult specimen of *A.dumerilii* with successful readable PIT tag identification through aquarium glass.

of sub adult and adult specimens marked with a PIT tag coincides with the rest period from reproduction in this species noted within captive collections to occur from April until September, where courtship begins, followed by spawning from October to March (Saldaña et al. 2006; Bland et al 2021). It would be expected that body weight may increase during this time of recovery from the previous breeding season, although speculative these results indicate that tagging did not interfere with this seasonal regaining of body weight.

Although sample size was limited due to the rarity of the species, PIT tagging in this instance was not associated with an increased mortality rate or found to cause any observed short or long-term negative health effects in captive *A. dumerilii*. This method of individual identification is beneficial to the management of captive colonies for conservation breeding, and could be utilised for mark recapture studies enabling a more detailed understanding of the wild population, its ecology and movements within Lake Pátzcuaro, facilitating the conservation of this species.

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