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Assessing Reliability of PIT-tagging in an Endangered Fossorial Toad (*Pelobates cultripes*) and its Effect on Individual Body Mass

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Abstract.—**Capture-mark-recapture studies that track individual amphibians are important for conservation, population dynamics, and ecological studies. One of the most widely used methods in wildlife marking is tagging with Passive Integrated Transponders (PIT). Tagging can adversely affect amphibians, however, and tag loss can affect the estimation of the demographic parameters. As part of a demographic survey for Western Spadefoot Toads (*Pelobates cultripes*), we used visual photo-matching to estimate the false negative rates (FNR) and PIT-tag retention rates (PTRR) of 101 PIT-tagged individuals (77 adults and 24 juveniles). We assessed the effect of PIT-tagging on changes in body mass between capture and recapture using comparison tests and Generalized Linear Model (GLM) analysis on 37 adults and 16 juveniles. The results show a PIT-tag retention rate of 100% for the 101 recaptured individuals regardless of the age group, and an overall false negative rate of zero. Maximum retention time observed was 238 d. The marked individuals did not lose mass between first capture and last recapture. During the monitoring period, adults did not lose mass, while juveniles gained mass significantly (6.87 g). The time effect was not significant for adults (GLM analysis) as their growth rate is slower than juveniles. There was a higher body mass gain in females than males, which could be explained by the accumulation of lipid stock (triglycerides). Our study shows that PIT-tagging was a very reliable method for *Pelobates cultripes* monitoring and that it did not lead to loss of body mass of individuals in the wild during the monitoring period.**

Key Words.—body mass variation; individual recognition; PIT-tag retention rate; scaled mass index; visual matching; Western Spadefoot Toad

Introduction

Probabilistic models and methods that are used to understand animal population dynamics have received considerable attention over the last few decades (Chao 2002; Cam 2009). Capture-mark-recapture (CMR) is one of the most commonly used techniques as it provides accurate demographic parameters (i.e., population size, survival rate, etc.), necessary to develop a relevant conservation strategy (Williams et al. 2002). The CMR method involves marking individuals with a permanent identifier that is easy to detect and univocal and does not negatively affect survival of the animal (White et al. 1982). Passive Integrated Transponder tags (PIT-tags) using Radio Frequency Identification (RFID) technology is one of the most used methods in wildlife marking (e.g., small mammals, reptiles, amphibians, birds, fishes) as it provides highly reliable individual marks (i.e., high PIT-tag retention rate) and has shown no long-term negative effects (Elbin and Burger 1994; Gibbons and Andrews 2004; Skov et al. 2020).

Failure of PIT-tags can unfortunately occur. The microchip injected into the coelomic cavity or subcutaneously may get lost through the open wound after the injection, through the body wall, or through the intestine (Jepsen et al. 2002). Loss of markers over time can lead to false negative errors due to failure to identify recaptured individuals, with consequent repercussions on the reliability of the estimated demographic parameters (Morrison et al. 2011; Johansson et al. 2020). Additionally, even though most studies have not shown long-term negative physiological effects of PIT-tagging, growth and survival disorders have been observed in multiple fish species (Baras et al. 2000; Ruetz et al. 2006; Tiffan et al. 2015). Furthermore, the negative effects of PIT-tags could be mitigated during pilot studies, which are mostly conducted under controlled conditions (i.e., in laboratory).

Various studies, which included both laboratory and field data, have shown different results with several species of animals (Calisi and Bentley 2009). Overall, these findings suggest that the reduction of movement,

the perturbation of the immune functions, and the inhibition of specific biological traits (e.g., biphasic activity of amphibians, fossorial behavior) during captivity experiments could sometimes minimize unwanted PIT-tagging effects. In the same way as fishes, the effects of tagging amphibians in the wild are poorly known given that 78% of studies (15 of 19) were conducted in captivity (see Appendix). Consequently, field experiments seem essential to evaluate objectively the validity of marking methods. We evaluated, under natural conditions, the effectiveness of PIT-tagging as an individual marking method for the Western Spadefoot Toad (*Pelobates cultripes*). To achieve this, during the pilot year of a CMR demographic survey, we assessed (1) the false negative rate of marked individuals, (2) the PIT-tag retention rate, and (3) the effect of this marking method on individual body mass.

Material and Methods

Studied species.—*Pelobates cultripes* is predominantly distributed in the Iberian Peninsula (Spain, Portugal) but also occurs in southern France ranging from the Atlantic coast to the Var Department in the Mediterranean region (Lizana et al. 1994; Lescure and Massary de 2012). Its snout-vent length (SVL) does not exceed 125 mm (Marangoni and Tejedo 2008). *Pelobates cultripes* is a mainly nocturnal amphibian that uses sandy or gravelly soil to burrow in during periods of adverse conditions (Recuero 2010). Due to its low dispersal capacity, *P. cultripes* is especially vulnerable to the degradation and fragmentation of its natural habitats (Gutiérrez-Rodriguez et al. 2017). This species is listed as Vulnerable (VU) at the global level on the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN; International Union for Conservation of Nature/Species Survival Commission Amphibian Specialist Group 2020).

Population sampling and individual marking.—We conducted this study in southeastern France in the municipality of Oppède (43°50′43″N, 5°10′10″E, department of Vaucluse). The study area covered 14.5 ha and consisted of a breeding pond surrounded by agricultural land. The cultivated part of the farmland was characterized by wine and lavender cultivations on a loose soil that favors the persistence of a large population of *P. cultripes*. We organized two fieldwork phases: one from the end of winter to the spring (from 6 March to 23 April 2019) and a second in autumn (from 23 September to 4 November 2019) as part of a demographic survey. Using headlights to locate toads, we caught individuals by hand during 10 nocturnal sessions. During each session, four or six observers systematically surveyed the entire study area during 135 or 90 min, respectively, which corresponded to the time needed to examine the whole site. We determined the sex of adults based on the presence of a glandular pad on the dorsal surface of the arm in males (Eggert and Guyétant 1999). We measured snout vent length (SVL) using a dial caliper (Wiha dialMax, precision of 0.1 mm; Wiha, Schonach im Schwarzwald, Germany) and body mass using a spring scale (100 g, precision of 0.3 g; Pesola, Schindellegi, Switzerland). We defined two age classes on the basis of skeletonchronological analysis by Leclair et al. (2005), which considers mature adults to be those individuals having a SVL ≥ 40 mm and juveniles as those with a SVL ranging from 34 to 39 mm. We marked and took photographs of all individuals ≥ 34 mm. Our database included adults ≥ 43 mm SVL and juveniles 34–39 mm SVL. Individuals < 34 mm SVL were too small to mark.

We marked captured individuals with RFID transponders of 1.4 × 9 mm (Standards, 134.2 khz; Biolog-ID SAS, Bernay, France). The mass of PIT-tags was 0.03 g, representing 0.09% (range, 0.04–0.60%) of the average body mass of toads. We inserted PIT-tags with sterile single-use needles below the skin from the internal face of the thigh to the outside alongside the femur to avoid vital organs (Fig. 1). We checked PIT-

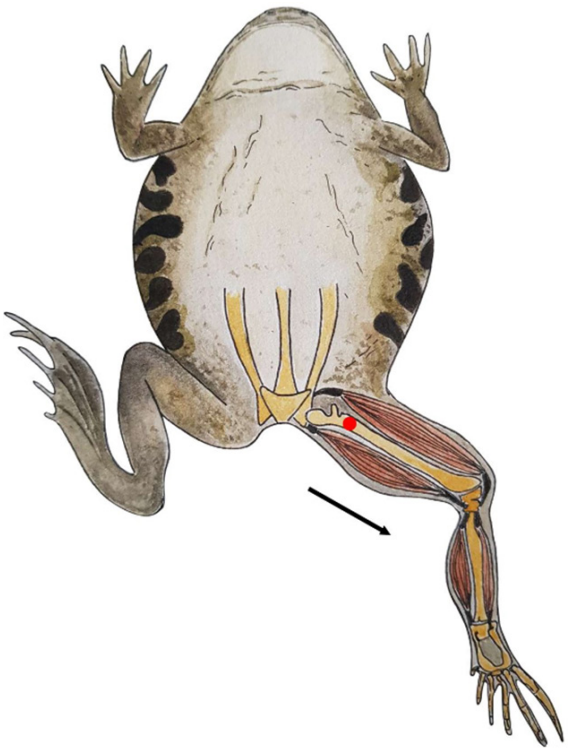

Figure 1. Ventral side of a Western Spadefoot Toad (*Pelobates cultripes*). Red dot represents the insertion area of the needle for the implantation of the PIT-tag under the skin and the arrow indicates the direction of needle insertion. (Illustration by Amanda Xérès).

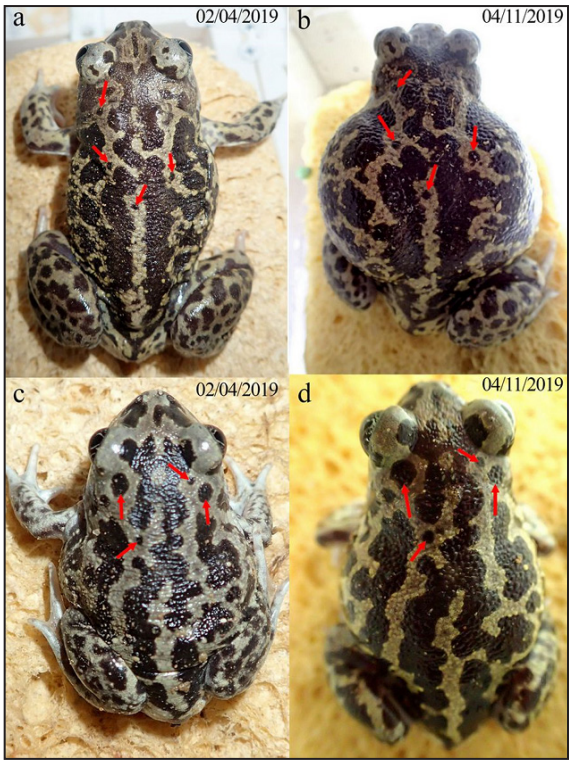

Figure 2. Visual matching used to assess the PIT-tag retention rate of Western Spadefoot Toads (*Pelobates cultripes*). First capture (a) and last recapture (b) of an adult female (PIT-tag ID: 4631445), about 7 mo apart. First capture (c) and last capture (d) of a juvenile individual (PIT-tag ID: 4631337), about 7 mo apart. Red arrows indicate part of the distinctive characters. Date format is day/month/year. (Photographed by Julien Renet).

tags within toads with RFID reader RS100 V8 (Biolog-ID SAS, Bernay, France). We performed photo-marking with a camera with a pixel resolution of 2400 × 3200 (model TG-3, Olympus, Tokyo, Japan). We positioned individuals on a wet sponge in a closed box with two holes, one for a Light-Emitting Diode (LED) lamp to illuminate the individual, and another one for the camera. We followed this protocol to increase the photographic standardization (i.e., photograph quality) and avoid an excessive manipulation of the amphibians (Fig. 2). We systematically photographed all captured and recaptured individuals during field sessions.

Assessment of false negative rate and PIT-tag retention rate.—To evaluate false negative rate (FNR) and PIT-tag retention rate (PTRR) to test the reliability of PIT-tagging in *P. cultripes*, we used photo-identification by visual matching of dorsal pattern (Kenyon et al. 2009; Ferner 2010; Smith et al. 2018), which is characterized by unique spots for each toad (Fig. 2). This method of individual identification has been successfully used with the congeneric species Common Spadefoot (*P. fuscus*), whose dorsal pattern displays the same characteristics as that of *P. cultripes* (Jehle and Hödl 1998). The FNR measures the probability that a recapture event is wrongly classified as a new capture due to a potential loss of a PIT-tag. This rate is defined as the ratio between the number of false negatives and the total number of identification attempts (Sacchi et al. 2016). The PTRR is the proportion of recaptured individuals that kept their PIT-tag.

To detect missing tags, we created a database of 372 theoretically unique photographs of marked individuals (239 adults and 133 juveniles) identified by PIT-tags. To detect potential allocation errors in the database (i.e., a tagged individual is considered as a new capture, although it was previously captured, prior to the loss of its tag), two experienced observers (GR and JR) visually compared the images one by one independently (i.e., all unique individual photographs were visually compared to all other photographs in the database). To evaluate the PTRR, the same two observers visually compared the images of 101 recaptured individuals (77 adults and 24 juveniles) to the 372 individuals of the database. We also calculated the maximum retention times of PIT-tags, counting the number of days between the first capture and the last recapture.

Assessment of the effect of marking on body mass.—To test the effect of PIT-tagging on the physiological state of the studied individuals, we focused on body mass, which represents an important fitness component (Peig and Green 2009). We restricted the analysis on adults to autumn data (23 September to 4 November 2019) to reduce bias due to egg production, which involves a mass gain for females during spring. The breeding period in *P. cultripes* usually extends from February to April (Gutiérrez-Rodriguez et al. 2017) and is characterized by body mass variations due to the investment for breeding as is common for most amphibians (Kuramoto 1978). We only considered adult individuals captured for the first time in autumn (15 males and 22 females), but we used the collected data on juveniles (16 individuals), which are sexually immature, for the entire study period. For each individual, we compared measurements (SVL and body mass) from first to last capture to examine the effect of PIT tagging over the longest period. At the time of the first capture, the average initial body mass was 33.3 g for males, 52.47 g for females, and 8.66 g for juveniles.

Standardization of body mass data.—To standardize and analyze body mass data, we used the scaled mass index (SMI) a body condition index based on the relationship between body mass and a linear predictor of body size, accounting for allometric growth (Peig and Green 2009) and which is computed as follows:

$$\widehat{M}i = Mi\left[\frac{L0}{Li}\right]^{b_{SMA}}$$

where M is the mass of the individual i and L is the SVL of individual i. $L0$ is the mean SVL value of the study population; b_{SMA} is the scaling exponent estimated by the SMA regression of M on L from the linearized power equation $ln\ M = ln\ a + b\ (ln\ L)$; is the predicted body mass for individual i when the linear body measure is standardized to $L0$. Among many condition indices, SMI has been shown to be more reliable than other methods in many animal taxa, in particular those based on residuals obtained from regressing body mass on body length (Peig and Green 2009, 2010; Kraft et al. 2019). In the specific case of amphibians, the SMI can be considered a robust index of the overall energy reserves and health condition of individuals (MacCracken and Stebbings 2012; Brodeur et al. 2020).

Mass variation statistical analyses.—To identify difference between first capture and last recapture mass, we used the calculated SMI values for each individual for all the analyses. First, we used the Shapiro-Wilk (SW) test to check normality of samples. To test whether individuals of the same sex (i.e., intra-sex) had lost or gained mass significantly, we performed paired t-tests (or Wilcoxon tests when Shapiro-Wilk normality test failed; Blair and Higgins 1985; Yue and Pilon 2004). We tested parametric assumptions of all statistical tests we used and assumptions were met. We tested all analyses for significance at $\alpha = 0.05$. To study the mass difference (i.e., body mass of the last recapture – body mass of the first capture) for males, females, and juveniles and to determine if the number of days between captures and recapture affected this variable, we used two Generalized Linear Models (GLM; Glonek and McCullagh 1995) with a Poisson distribution and log link function on the dataset. The first model tested the effects of inter-sex and number of days on adult body mass between capture and recapture, while the second GLM tested the effect of number of days on juveniles. For each GLM, we used Goodness-of-Fit tests (GoF) to verify if the models fitted to the data, calculating the dispersion parameter and the Pseudo-R2. We performed a Chi-squared Test to check if the overdispersion or underdispersion was significant and, in the case of overdispersion, we used negative binomial distribution for the two models, following the same path for the tests of GoF.

We performed all analyses in the software R. 3.6.0 with the interface RStudio (R Development Core Team 2018). We calculated the scaling exponent b_{SMA} directly by using the Smart package (Warton et al. 2012). We used the other functions in the packages MASS (Venables and Ripley 2002) and Stats (Chambers and Hastie 1992).

Results

PIT-tag retention and false negative rate.—We did not detect any change concerning the body patterns of the compared adults (Fig. 2), while we found an evolution of body patterns of juveniles, although they were still easily recognizable (Fig. 2). The visual comparison of the 372 marked individuals revealed a PTRR of 100% (on 101 recaptured individuals) and a FNR of zero. These results indicate that no individual was tagged multiple times (i.e., had lost a first PIT-tag and was re-marked). The 77 adults and the 24 juveniles recaptured at least once during the year conserved their PIT-tags over the entire study. The maximum known tag retention time was 238 d (i.e., near 8 mo).

Body-mass changes.—Body mass index was normally distributed for both males and females at first and last capture (Shapiro-Wilk test, males captured: SW = 0.92, P = 0.211; males recaptured: SW = 0.98, P = 0.985; females captured: SW = 0.96, P = 0.594; females recaptured: SW = 0.97, P = 0.805). By contrast, body mass was not normally distributed for juveniles at either point in time (juveniles captured: SW = 0.88, P = 0.041; juveniles recaptured: SW = 0.91, P = 0.133). Body mass did not differ significantly between first and last capture for females (t = 1.94, df = 21, P = 0.067; Fig. 3) or males (t = 0.84, df = 14, P = 0.417; Fig. 3). The mean difference

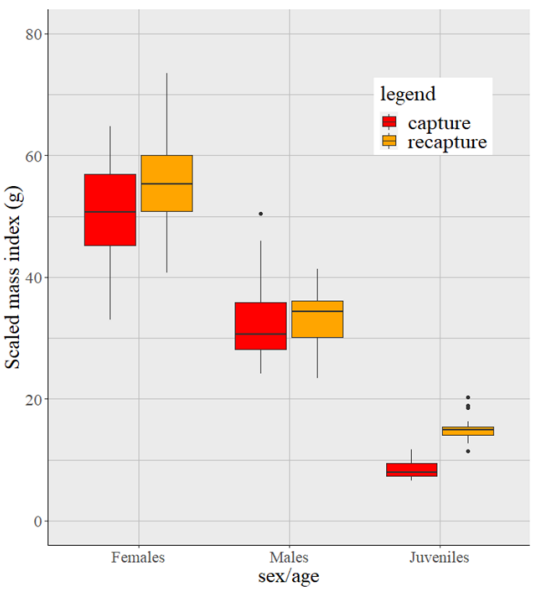

Figure 3. Boxplots indicating body mass variation of Western Spadefoot Toad (*Pelobates cultripes*) between the first capture and the last recapture for the three considered groups (females, males, and juveniles). The vertical axis represents body mass estimated with the SMI method (see Material and Methods). The box for each category represents the interquartile range and the central line within the box represents the median. Vertical lines are the 95% confidence intervals and dots are outliers.

Table 1. Generalized Linear Model analysis of the mass differential between capture and recapture in adults and juveniles of Western Spadefoot Toad (*Pelobates cultripes*). Analysis I, assessment of adult mass differential varies as a function of sex and number of days between capture and recapture. Analysis II, assessment of juvenile mass differential varies as a function of number of days between capture and recapture. Abbreviations are SE = standard error; Sex = sex of adult individuals; Day = number of days between capture and recapture; Intercept = predicted value of the dependent variable when all independent variables are 0. Bold font indicates significant *P* values.

Analysis	Parameter	Estimation	SE	*P*	Dispersion parameter φ	Pseudo-R2
I	Intercept	0.93849	0.3464	< 0.007	1.20; *P* > 0.190	0.17
	Sex	-0.6921	0.3104	**< 0.026**		
	Day	0.0212	0.0143	> 0.139		
II	Intercept	1.0200	0.3381	< 0.003	1.31; *P* > 0.192	0.34
	Day	0.0060	0.0020	**<0.002**		

in body mass was 3.39 g (± 1.751 g standard error) for females and 1.35 g (± 1.621 g) for males. Body mass of juveniles increased significantly (V = 136, $P < 0.001$, n = 16) with a mean difference of 6.87 g (± 0.691 g) between first and last capture (Fig. 3).

Effect of sex and time on mass difference.— The average time between the first capture and the last recapture was 19.02 d (± 10.06 d) for adults and 134.19 days (± 89.30 d) for juveniles. For adults, the effect of time was not significant (Table 1, Analysis I). Conversely, there was a considerable negative sex effect for males given they had a mass difference smaller than females (Table 1). For juveniles, time effect was significant (Table 1, Analysis II). The mass differential increased as the time between capture and recapture lengthened and was related to the growth of individuals. This model was adjusted to the data and explained 34% of the data (Table 1, Analysis II).

Discussion

With 100% PIT-tag retention, we found high reliability of this marking technique for *P. cultripes* monitoring. In anurans, estimation of PIT-tag retention rate varies between 33% and 100% (Brown 1997; Jehle and Hodl 1998; Blomquist 2008; Brannelly et al. 2014) and between 55% to 100% in urodelans (Unger et al. 2012; Ousterhout and Semlitsch 2014; Ryan et al. 2014; Whiteman et al. 2016; Le Chevalier et al. 2017). These retention rates must be interpreted carefully, however, because some of them were calculated on a reduced sample of individuals and most were obtained under controlled conditions.

Among anurans, several factors can affect retention rate, including the site of PIT-tag insertion and the skin texture of target species. For example, in 20 Northern Leopard Frogs (*Rana pipiens*), Blomquist (2008) obtained 100% of PIT-tag retention for scapular insertion while this rate decreased to 90% and 55% for ilium and pubis insertion, respectively. Variation of retention rate was also observed by Brannelly et al. (2014) in Alpine Tree Frogs (*Litoria verreauxii alpina*), with 33.3% tag retention for an implantation in the body cavity and 73.3% for subcutaneous injection into the left axillary region. Among urodelans, PIT-tags are frequently inserted in the ventral posterolateral abdominal wall, which might explain the less strong variations of PTRR among studies. The study of *Litoria verreauxii alpina* also showed that PIT-tags can migrate through the skin far from the incision mark, leading to expulsions (Brannelly et al. 2014). This may mean that the texture of the skin also has an influence. According to Unger et al. (2012), the tag has a higher probability of being expelled in the days following the implantation, before the complete healing of the incision site. As for most terrestrial amphibians, the skin of *P. cultripes* is thick and compact (Toledo and Jared 1993) and the healing process is fast. These two characteristics probably contributed to the high tag retention in this species, together with the choice of inserting the needle as far as possible from the incision zone parallel to the femur to reduce the risks of expulsion.

Concerning the monitoring of the physiological state of *P. cultripes*, PIT-tagging did not cause body-mass loss in adults and juveniles during the entire survey. In adults, the mass difference was lower as their growth is slower than juveniles and, consequently, it becomes difficult to discern changes in their body condition. Indeed, these results seem to indicate that tagging probably caused a low energetic cost to *P. cultripes* and minimal effects on its behavior and physiology. Similarly, studies on other amphibian species have shown a low energetic cost of PIT tagging (see Christy 1996; Pyke 2005; Schulte et al. 2007; Connette and Semlitsch 2012). The average mass gain observed of *P. cultripes* adults during autumn could be explained by the accumulation of lipid stock (triglycerides) for metabolic maintenance during winter dormancy (Fitzpatrick 1976). In our study females gained more body mass than males in relation to higher energetic requirements during the breeding season

(egg-laying, displacements on breeding pond, etc.; Jorgensen 1992; Vimercati et al. 2019). Ontogenetic growth during the studied time can explain the stronger differences between the capture and recapture body masses of juveniles. In juveniles, the increased body mass is significant with an average mass gain of almost 7 g. Nevertheless, the lack of a control group does not permit us to infer if this gain corresponded to standard development.

Our results demonstrate that PIT-tagging is an efficient marking technique, with minor impacts, for monitoring *P. cultripes*. The manipulation during the insertion of transponders surely causes stress, but the procedure is fast (3 min on average) and occurs only once because tag retention is high. Further, tags can be remotely detected (approximately 10 cm) without catching the animal. To reduce infection risk and pathogens transmission, we recommend inserting the tag on the internal face of the thigh along the femur with sterile single-use materials.

The development of photo-identification for wildlife monitoring (manual or automated; see Matthé et al. 2017; Renet et al. 2019; Gould et al. 2021) encourages us to recommend this individual marking method. The employment of this technique in a long-term survey of *P. cultripes* appears to be unsuitable, however, because (1) the undefined evolution of dorsal pattern throughout multiple years implies possible misidentification, (2) the systematic manipulation of animals to ensure standardization of photographic parameters (e.g., light, angle) could stress them, and (3) automated photo-identification is not always usable depending on photograph quality, while visual matching is much more time-consuming and more liable to allocation errors due to the interobserver variability (experienced versus non-experienced; Cruickshank and Schmidt 2017). This option, however, may be valuable in case the budget does not allow the purchase of tags, low densities (i.e., reduction of error rate and photographic treatment) and, above all, in case there is no doubt regarding the immutability of dorsal pattern.

Finally, in the context of an amphibian CMR study through PIT-tagging, we suggest long-term supervision of marked animals because the emergence of wounds and disorders due the migration of microchip may occur. Indeed, we believe that the time frame of this study, which is probably too short to rule out any risk of foreign body complications, is sufficient to robustly assess the PTRR (low risk of rejection after wound healing). For this reason, it is necessary to regularly measure SVL, body mass, or other proxies to monitor the evolution of body condition of the individual.

This study is a further contribution to an evaluation of PIT tagging in wild tracked amphibians. We encourage biologists to evaluate the reliability and effects of marking devices used to study populations of wildlife species. This is essential to improve technological tools and minimize negative impacts on wildlife.

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Appendix Table. Review of experimental conditions and parameters considered to assess the PIT-tag retention rate (PTRR) and physiological effects (PE) of PIT-tagging in different urodelan and anuran studies. Species listed are Italian Crested Newt = *Triturus carnifex*, Alpine Newt = *Icthyosaura alpestris*, Danube Crested Newt = *Triturus dobrogicus*, Marbled Salamander = *Ambystoma opacum*, Fire Salamander = *Salamandra salamandra*, Red-legged Salamander = *Plethodon shermani*, Southern Gray-cheeked Salamander = *Plethodon metcalfi*, Eastern Hellbender = *Cryptobranchus alleganiensis*, Ringed Salamander = *Ambystoma annulatum*, Blue-spotted Salamander = *Ambystoma laterale*, Arizona Tiger Salamander = *Ambystoma tigrinum nebulosum*, Marbled Newt = *Triturus marmoratus*, Black-bellied Salamander = *Desmognathus quadramaculatus*, Seal Salamander = *Desmognathus monticola*, Great Crested Newt = *Triturus cristatus*, Striped Marsh Frog = *Limnodynastes peronii*, Common Frog = *Rana temporaria*, Common Toad = *Bufo bufo*, Common Spadefoot = *Pelobates fuscus*, Golden Bell Frog = *Litoria aurea*, Northern Leopard Frog = *Rana pipiens*, Alpine Tree Frog = *Litoria verreauxii alpina*.

	Species	Captivity	Field condition	PTRR	PE	Sample size	Duration	References
Urodela	*Triturus carnifex*	X		X	X	11	5.5 mo	Fasola 1993
	Icthyosaura alpestris	X		X	X	12	5.5 mo	Fasola 1993
	Triturus dobrogicus		X	X	X	551	9 y	Jehle and Hoedl 1998
	Ambystoma opacum	X		X	X	260	6 weeks	Ott and Scott 1999
	Icthyosaura alpestris	X	X	X	X	180 (captivity), 121 (field condition)	2 mo (captivity), 5 d (field condition)	Perret and Joly 2002
	Salamandra salamandra	X		X	X	10	2 y	Schulte et al. 2007
	Plethodon shermani	X		X	X	18	9 weeks	Connette and Semlitsch 2012
	Plethodon metcalfi		X	X		6	9 d	Connette and Semlitsch 2012
	Cryptobranchus alleganiensis		X	X		78	2 y	Unger et al. 2012
	Ambystoma annulatum	X		X		27	6 weeks	Ousterhout and Semlitsch 2014
	Ambystoma laterale	X		X		532	2 y	Ryan et al. 2014
	Ambystoma tigrinum nebulosum	X		X	X	20	10 d	Whiteman et al. 2016
	Triturus marmoratus	X		X	X	46	70 d	Le Chevalier et al. 2017
	Desmognathus quadramaculatus	X		X		8	37 d	Mitchell et al. 2017
	Desmognathus monticola	X		X		24	260 d	Mitchell et al. 2017
	Triturus cristatus		X	X	X	100	9 d	Weber et al. 2019
Anura	*Limnodynastes peronii*	X		X	X	6	1 mo	Christy 1996
	Rana temporaria	X		X	X	5	22 mo	Brown 1997
	Bufo bufo	X		X	X	30	8 mo	Brown 1997
	Pelobates fuscus		X	X	X	1220	8 y	Jehle and Hoedl 1998
	Litoria aurea		X		X	2950	6 y	Pyke 2005
	Limnodynastes peronii		X		X	294	6 y	Pyke 2005
	Rana pipiens	X		X	X	102	2 weeks	Blomquist 2008
	Litoria verreauxii alpina	X		X	X	15	6 weeks	Brannelly et al. 2014