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Marking techniques in the Marbled Newt (*Triturus marmoratus*): PIT-Tag and tracking device implant protocols

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Abstract. Individual marking has become essential for studying population dynamics and ecological requirements. However, marking small-bodied species such as amphibians is becoming a challenge in the last decades. Amphibian surveys may require to mark manually individuals, using toe clipping, polymers and pigments, or passive integrated transponders (PIT-tags). Even if ethics committees have recently recommend avoiding toe clipping in amphibians, the use of PIT-tags led to controversial results because low tag retention reported in some studies. Here, we describe a protocol of potentially life-long PIT-tag marking in a protected species, the marbled newt *Triturus marmoratus*. In addition, we also detailed a second procedure of surgery for the implantation of transmitters needed in radio-tracking surveys. During both procedures, we found that the newt phase (either aquatic or terrestrial) strongly affected the anesthesia duration. Indeed, newts in aquatic phase were more quickly anesthetized than newts under terrestrial phase. We then recommend to pay attention of this physiological particularity when performing this kind of procedure. Improving our knowledge on ecological requirements and population dynamics of this species is crucial for management and conservation plans, and could be extended to other large newts.

Keywords. Anesthesia, transmitter implantation, *Triturus marmoratus*, PIT-tagging, newts, skin permeability

INTRODUCTION

Individual marking has become an essential method for studying ecological requirements, population dynamics or colonization rates (McCarthy and Parris, 2004). Two different methods are widely used for monitoring species: capture-marking-recapture (CMR) and radio-tracking surveys. The CMR method is a powerful method for estimating population parameters such as abundance (Thompson et al., 1998), survival, recruitment, and population growth rate (Lettink and Armstrong, 2003). For CMR studies, marking of individuals can be performed using several techniques (Ferner, 2007), such as color pattern (non-invasive method often used in some salamanders and anurans species; see Delarze et al., 2000; Lama et al., 2011; Ribeiro and Rebelo, 2011; Waye, 2013; Elgue et al., 2014), the use of passive integrated transponders (PIT-tags; Jehle and Hödl, 1998), toe clipping, or the use

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of polymers and pigments (Brannelly et al., 2014). Toe clipping is a technique commonly applied to amphibians, but may be useful for a limited time due to their regeneration ability (Andreone, 1986). Indeed, time intervals for regeneration range between less than one year to several years or not observable regenerations, depending on the species (Ferner, 2007). This technique could also be a source of stress and involves tissue damage and a risk of infection in many species and may also interfere with behaviour and movement pattern of individuals (Parris and McCarthy, 2001; McCarthy and Parris, 2004; Funk et al., 2005). Photo matching is a relatively low-costly and non-invasive powerful technique allowing individuals to be recognize using photographic identification of external body markings (Arntzen et al., 2004). This technique had been used in a variety of species, including newts (Drechsler et al., 2015; Mettouris et al., 2016; see also Diego-Rasilla and Luengo, 2002) but photo-identification might be difficult to apply in some cases (for instance in the marbled newt during the aquatic phase, females are very dark colours with very low contrast). Observers can correctly match 100% of photo pairs "by eye" (Morrison et al., 2016), but this process is highly time-consuming, and was considered as relatively not appropriate for studies on large populations (Arntzen et al., 2004). Since several years, many automatic recognition softwares have been developed (Wild-ID, Bolger et al., 2012; APHIS, Moya et al., 2015; Hotspotter, Crall et al., 2013; AMPHIDENT, Matthé et al., 2008; I^3S Pattern, van Tienhoven et al., 2007) to reduce the time of individual identification. Many studies demonstrated that, using automated processes for photographic re-identification, pattern mapping is a successful approach for the identification of individuals, even in large populations (Drechsler et al., 2015; Mettouris et al., 2016). However in some cases, these tools failed to match many image pairs (Morrison et al., 2016), and might induce significant bias in the CMR analysis.

PIT-tagging is a relatively new marking technique (Christy, 1996) providing rapid recognition of individuals during recapture sessions and limiting recognition errors (using recorder scanning device for instance). Also, some scanning devices can read the PIT-tags at several centimeters of the marked individual, which reduce the stress induced during the manipulation. Along with the demonstration of drawbacks of this method in amphibians (Tracy et al., 2011; Brannelly et al., 2014), such as low tag retention among marked individuals and the expense, ethics committees have recently recommend avoiding toe clipping in amphibians. Indeed, other recent studies showed pertinent results using PIT-tagging for amphibian monitoring (Connette and Semlitsch, 2011; Heard et al., 2012) with no significant effects of marking on survival rate or body condition (Perret and Joly, 2002). Miniaturization process of PIT-tags and similar marking techniques has increased these last years, being less invasive, and the size should continue to decrease in the future (Gibbons and Andrews, 2004; Cooke et al., 2013).

PIT-tagging (relatively costly and rapid recognition) and photo identification (low-costly and non-invasive) are often compared for studying the benefits and disadvantages of each technique (Arntzen et al. 2004), and to identify the more appropriate method to apply on amphibian populations. The choice of technique depends on financial and human costs, but also on the study species, study duration and sampling proportion (Arntzen et al. 2004). The use of photo-identification technique is well documented in the literature (Drechsler et al., 2015; Mettouris et al., 2016; Sannolo et al., 2016) but clear and detailed protocols about the use of PIT-tagging still remain rare, in particular in newts. Moreover, it is noteworthy that individuals tested here were captured for locomotion and mobility experimental tests under controlled conditions (see below) and that each individual from host captive wildlife within French establishments authorized must be marked with PIT-tagging since August 2004 (art. R. 413-30; 10 August 2004).

Radio-tracking surveys are also commonly used in many species, even in small-bodied urodeles (Jehle and Arntzen, 2000; Ribéron and Miaud, 2000; Rittenhouse and Semlitsch, 2006). For radio-tracking studies, an active transmitter is either attached to the different parts of the animal body, depending on the taxon (stuck on carapace, neck or wings) or surgically implanted into the coelomic cavity. Then, tracked individuals can then be located using a receiver. Contrary to CMR surveys, radio-tracking allows precise and remote detection of individuals allowing determination of habitat use and daily movements.

Here, we describe the protocol of a successful PIT-tag marking in the marbled newt. We also detail a surgical procedure for the implantation of transmitters in this species. Details on individual morphology, anesthesia duration, and the effect of individual phase (terrestrial or aquatic phase) are explored.

Studies on ecological requirements and population dynamics of this species are crucial for management and conservation plans. Technological progress over the last few years in transmitter size allows the use of small transmitters without an external part. Developing an efficient protocol for both PIT-tagging and implantation of transmitters could strongly be useful and applicable in other large newt species.

MATERIALS AND METHODS

Study species

The marbled newt (*Triturus marmoratus*) is a protected species listed on both the Bern Convention and the European Habitat Directive (annex III and annex IV respectively; least concern on the IUCN RedList), with declining populations (Arntzen et al., 2009). During the breeding season (from March to May), this species lives in a variety of temporary or permanent water sources, such as well-vegetated ponds, pools, ditches and streams (Nöllert and Nöllert, 2003). Newts have a permanent permeable skin. But during the breeding season their skin is more permeable, allowing for respiration, but also resulting in significant water loss. A nearby water source therefore constitutes critical habitat for this species during reproduction (Wells, 2007). After breeding, the skin of newts undergoes a seasonal change in osmotic permeability and becomes less permeable (Wells, 2007). Newts become more terrestrial and move out of water to winter and feeding habitats, notably wet habitats such as forests or under stones, to hibernate. Within this terrestrial phase, the mobility of the marbled newt is estimated to be less than 1 kilometer (Jehle, 2000; Jehle and Arntzen, 2000).

Newt sampling

We captured a total of 46 marbled newts (22 females and 24 males) from two sites to minimize the impact on newt populations and also to test if radio-tracking survey data were similar between individuals from different landscapes (see Trochet et al., 2017). We sampled marbled newts in southern France, in the department of Ariège (n = 30; 12 females and 18 males; 43.076347°N, 1.351639°E) between the 1st and the 29th April 2015, and in the department of Gers (n = 16; 10 females and 6 males; 43.671781°N, 0.504308°E) between the 24th April and the 6th of May 2015. Individuals were caught using a landing net and transported to the Station d'Ecologie Théorique et Expérimentale CNRS (42.958285°N, 1.086455°E; Moulis, FRANCE), an ecological research station of the National Center for scientific research situated in the foothills of the Pyrenees. Animals were housed in groups of 6 to 8 individuals in aquaria of 60×30×30 cm and kept at a temperature of 20°C, and were fed with mealworms. For each individual, we measured the snout-vent length (SVL) using a caliper and the body mass (BM) with an electronic scale (precision 0.01 g).

PIT-tagging was performed between the 2nd April and the 11th May 2015, during the aquatic phase of the breeding season when newts had large crest in males and highly permeable skin (Table 1). During this aquatic phase, the skin is smoother and less coloured. Transmitter implantations were then performed between the 28th May and the 19th June 2015 during the post-breeding migration when newts were in the terrestrial phase, recognizable by the small crest in males and low permeable skin (individuals more coloured and grainy skin; Table 1).

All individuals were pit-tagged, with 24 individuals also implanted with transmitters. Animals were released after 40 to 70 days at their site of capture after locomotion and mobility experimental tests (data not shown here).

Anesthesia procedure

Both procedures were performed in sterile conditions using diluted Chlorexidine 0.75%. Prior to each procedure, individuals were rinsed and placed in individual boxes. Considering the average weight of newts (mean ± SD: 11.53 ± 3.32 g; min-max: 5.54 – 20.23 g; Table 1) and the concentration of Lidocaine/Prilocaine in EMLA ointment (5% Lidocaine 2.5% and Prilocaine 2.5%; AstraZeneca GmbH Laboratories, Germany, EMLA), the final dosage for anesthesia was 450 mg/Kg, by percutaneous absorption of the ointment in a cutaneous squared surface of 1cm × 1 cm. We applied one spot of cutaneous anesthetic cream on their left flank, until the muscular system was relaxed and animals stopped moving. We considered animals to be surgically anesthetized (only cardiac impulse was present) when individuals lost the "withdrawal reflex" (i.e. no response when pinching digits and tail; Fig. 1a) and "righting reflex" (i.e. unable to right themselves when put on their back; Fig. 1b; Mitchell, 2009). Animals were rinsed before the procedures to remove excess cream. Anesthesia and recovery durations were recorded for both protocols.

PIT-tagging protocol (n = 46)

During anesthesia, the newt skin was firstly disinfected before surgery with diluted Chlorexidine 0.75%. We then placed a PIT-tag (RFID Standards ISO 11784 & 11785 type FDX-B, 1.4×8 mm, 134.2 khz from BIOLOG-ID, FR; Fig. 1c) into the dorsal side using an injector, previously disinfected with diluted Chlorexidine 0.75%. The mean mass of PIT-tags was 0.03 g, representing approximately 0.26% (range min-max: 0.15-0.54 %) of the newt body mass. The needle was inserted on the left side, at the site of anesthetic application, from the bottom of the back and pushed up under the skin to install the PIT-tag lateral to the hepatic area between the posterior and anterior limbs. The injection site was immediately disinfected after injection with Chlorexidine 0.75%. PIT-tags were not removed from individuals before releasing.

Transmitter implantation (n = 24)

During anesthesia, a longitudinal incision of about 1 centimeter, matching the width of the transmitter, was made on the right flank using surgical scissors in two steps, for the skin and then muscle tissue. The transmitter (V1|10A ultimate lite implants: battery life around 54 days; BIOTRACK, UK) was placed into the abdominal cavity and fitted between the internal organs (Fig. 2a). The mean mass of transmitters was 1.77 ± 0.04 g, representing approximately 16% of the newt body mass (average 10.65 ± 2.23 g), a proportion considered unlikely to impact displacements (Madison and Farrand, 1998). The muscle tissue and the skin were then pulled over the transmitter and sutured. The muscle tissue was sutured with running subcuticular clo-

Table 1. Individual data on the 46 marbled newts used for both PIT-tagging and transmitter implantations. ID: individual number (corresponding to the seven last digits of PIT-tag number); SVL: snout-vent length (cm); BM: body mass (g).

Capture		PIT-tags		Transmitter implantations		Morphology			Release date
Site	Date	ID	Date	Transmitter frequency	Surgery date	Sex	SVL	BM	
Ariege	01/04/2015	3891004	02/04/2015	150.2431	01/06/2015	M	6.30	8.51	10/06/2015
Ariege	01/04/2015	3891021	02/04/2015	150.3037	02/06/2015	F	6.75	9.41	10/06/2015
Ariege	26/04/2015	3560269	26/04/2015	150.1231	02/06/2015	M	6.20	9.38	10/06/2015
Ariege	26/04/2015	3797561	26/04/2015	150.2730	02/06/2015	F	7.60	12.67	10/06/2015
Ariege	26/04/2015	3910400	26/04/2015			M	6.40	8.37	10/06/2015
Ariege	26/04/2015	3910360	26/04/2015	150.0941	01/06/2015	M	6.10	7.51	Dead
Ariege	28/04/2015	3560248	29/04/2015	150.0040	28/05/2015	F	7.80	14.69	08/06/2015
Ariege	28/04/2015	3793003	29/04/2015	150.2141	28/05/2015	M	7.90	14.75	08/06/2015
Ariege	28/04/2015	3563668	29/04/2015	150.0327	02/06/2015	F	7.05	10.73	08/06/2015
Ariege	28/04/2015	3793285	29/04/2015	150.1530	02/06/2015	F	7.00	12.99	08/06/2015
Ariege	28/04/2015	3910145	29/04/2015	150.1832	02/06/2015	M	7.00	9.20	08/06/2015
Ariege	28/04/2015	3560302	29/04/2015			F	6.50	9.16	08/06/2015
Ariege	28/04/2015	3910246	29/04/2015			M	7.30	12.00	08/06/2015
Ariege	28/04/2015	3560049	29/04/2015			M	6.90	9.90	08/06/2015
Ariege	28/04/2015	3793236	29/04/2015			M	7.75	14.57	08/06/2015
Ariege	28/04/2015	3910116	29/04/2015			M	7.10	10.31	08/06/2015
Ariege	28/04/2015	3560290	29/04/2015	150.3333	01/06/2015	F	7.45	11.95	10/06/2015
Ariege	28/04/2015	3910488	29/04/2015	150.7132	01/06/2015	M	7.30	11.38	10/06/2015
Ariege	28/04/2015	3560094	29/04/2015	150.0636	02/06/2015	F	7.70	13.69	10/06/2015
Ariege	28/04/2015	3560259	29/04/2015	150.7424	01/06/2015	M	7.10	10.33	Dead
Ariege	29/04/2015	3563674	30/04/2015			M	7.40	11.95	08/06/2015
Ariege	29/04/2015	3909734	30/04/2015			M	8.05	16.89	08/06/2015
Ariege	29/04/2015	3796489	30/04/2015			M	7.60	13.04	08/06/2015
Ariege	29/04/2015	3796891	30/04/2015			M	7.40	11.57	08/06/2015
Ariege	29/04/2015	3793419	30/04/2015			F	7.90	18.67	08/06/2015
Ariege	29/04/2015	3890964	30/04/2015			F	7.25	16.27	08/06/2015
Ariege	29/04/2015	3909958	30/04/2015			F	7.80	20.02	08/06/2015
Ariege	29/04/2015	3563699	30/04/2015			F	7.90	20.23	08/06/2015
Ariege	29/04/2015	3560116	30/04/2015			M	7.90	14.12	08/06/2015
Ariege	29/04/2015	3560253	30/04/2015			M	7.70	13.97	08/06/2015
Gers	26/04/2015	3560111	26/04/2015	150.772	18/06/2015	F	6.70	7.58	23/06/2015
Gers	26/04/2015	3910195	26/04/2015			F	6.70	10.29	23/06/2015
Gers	26/04/2015	3910288	26/04/2015			F	7.10	9.53	23/06/2015
Gers	26/04/2015	3910201	26/04/2015			M	6.75	8.67	23/06/2015
Gers	06/05/2015	3792718	11/05/2015	150.623	17/06/2015	M	6.90	9.61	23/06/2015
Gers	06/05/2015	3796526	11/05/2015	150.922	17/06/2015	F	7.55	14.39	23/06/2015
Gers	06/05/2015	3560285	11/05/2015	150.532	18/06/2015	M	6.60	9.47	23/06/2015
Gers	06/05/2015	3560266	11/05/2015	150.562	18/06/2015	F	6.65	11.12	23/06/2015
Gers	06/05/2015	3909919	11/05/2015	150.651	18/06/2015	M	6.40	8.93	23/06/2015
Gers	06/05/2015	3909854	11/05/2015	150.802	18/06/2015	F	7.15	10.60	23/06/2015
Gers	06/05/2015	3910535	11/05/2015	150.831	18/06/2015	M	6.10	10.09	23/06/2015
Gers	06/05/2015	3910390	11/05/2015	150.502	19/06/2015	F	6.60	7.96	23/06/2015
Gers	06/05/2015	3560126	11/05/2015	150.592	19/06/2015	F	7.20	8.63	23/06/2015
Gers	06/05/2015	3910153	11/05/2015			M	5.80	5.54	23/06/2015
Gers	06/05/2015	3563687	11/05/2015			F	7.10	12.23	23/06/2015
Gers	06/05/2015	3560239	11/05/2015			F	6.50	7.35	23/06/2015

Fig. 1. Anesthesia procedure after application of EMLA cream: loss of pain reflex (a); loss of righting reflex (b); PIT tag implantation (c).

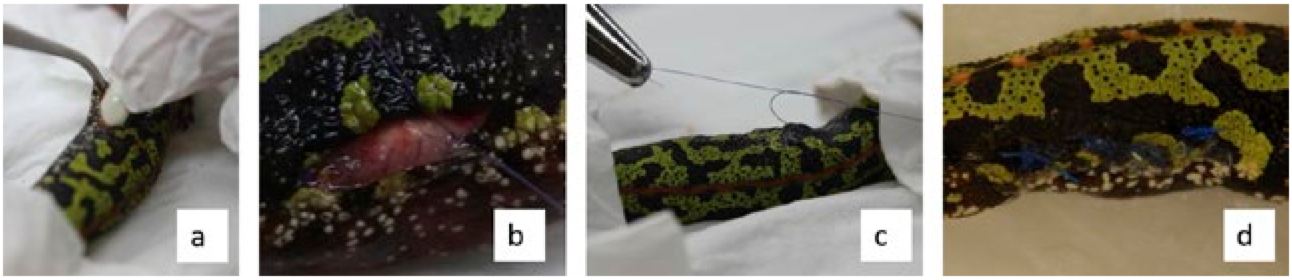

Fig. 2. Surgical implantation of transmitter: insertion of transmitter in the abdominal cavity (a); completed suture of muscle tissue using a simple continuous suture pattern (b); skin suture using the horizontal mattress suture technique (c); and completed procedure (d)

sure (Fig. 2b) using absorbable material (ETHILON, diameter 3/0). The skin was sutured with simple interrupted suture (3, 4 or 5 surgeon's knots depending on the length of the incision; Fig. 2c, 2d) using absorbable material (ETHILON, diameter 3/0). Sutures were performed using an iris cutting needle (a C-shaped needle 7 mm long) and surgical silk. Transmitters were not removed from individuals before releasing.

Recovery phase

After each protocol, anesthetized individuals were then placed in a recovery room, into wet boxes, where the ventral portion of the body was placed into water to promote the expulsion of the anesthetic agent. We considered newts to be recovered with the return of the righting reflex. They were then placed into a well-vegetated terrarium, with water, soil substrate, shelter (mud) and food (tubifex worms) and kept under observation for 5 to 10 days. No visible impact on health and behaviour was observed after the PIT-tagging procedure. However after transmitter implantation, triggering of a molt on 14 newts was observed two days after operation. The return of the feeding behaviour occurred in the four days after the operation.

Statistical analyses

We performed a total of 70 fully-recorded procedures (46 PIT-tag implants, 24 transmitter implants). In order to test if skin permeability (i.e., the date of both operations) could have an effect both on the anesthesia and the recovery durations (both not normally distributed), we used non-parametric Spearman correlation tests. We also compared both the anesthesia and the recovery durations and their variability (i.e., using a coefficient of variation defined as standard variation/mean × 100) depending on the two procedures tested here using Wilcoxon tests. We then calculated a condition index (hereafter CI) for each newt estimated by the residuals of the regression of log(BM) on log(SVL) (Jakob et al., 1996), a useful index in amphibian studies (Denoël et al., 2002; Bancila et al., 2010) to test if the morphology could be related to both anesthesia and recovery durations in the two different procedures using Spearman correlations. Finally, we tested if the sex had an influence on both anesthesia and recovery durations using Wilcoxon tests. All statistical analyses were performed using R 3.0.1. (R Development Core Team, 2014).

RESULTS

Anesthesia duration

Our results showed that the anesthesia duration was shorter among individuals in aquatic phase (mean ± SD: 5.17 ± 3.09 minutes) than among newts in terrestrial phase (mean ± SD: 10.15 ± 4.49 minutes) and that anesthesia duration was variable as both during PIT-tagging as in transmitter implantation procedures (Wilcoxon test: W = 1, P = 1). The anesthesia duration was significantly different between both procedures (Wilcoxon test: W = 125.5, P ≤ 0.01) and increased from the first procedures performed (i.e., PIT-tagging) at the beginning of April 2015 (aquatic phase with high permeable skin) to the final procedures (i.e., transmitter implantation) performed at the end of June 2015 (terrestrial phase with less permeable skin; Spearman correlation: $r_s = 0.39$, P < 0.01; Fig. 3).

We found non-significant relationship between the anesthesia duration and newts CI (Spearman correlations: $r_s = -0.08$, P = 0.50). Our results also showed no influence of sex on the anesthesia duration (Wilcoxon tests: W = 560.5, P = 1).

Recovery duration

Our results did not found difference in recovery duration variability between both procedures (Wilcoxon test: W = 0, P = 1). We found a strong difference in the recovery duration between the two procedures (Wilcoxon tests: W = 43, P < 0.01). The recovery duration after PIT-tagging (mean ± SD: 76.45 ± 27.51 minutes) was significantly shorter than the recovery duration after transmitter implantation (mean ± SD: 309.80 ± 87.93 minutes; Spearman correlation: $r_s = 0.60$, P = < 0.01; Fig. 4).

Contrary to the anesthesia duration, newt CI influenced recovery duration (Spearman correlations: $r_s = -0.27$, P = 0.02) where the largest newts woke up faster. Our results showed no influence of sex on the recovery duration (Wilcoxon tests: W = 635, P = 0.36).

PIT-tags and transmitter surveys

No PIT-tags were reported lost, even several months after implantation. Before releasing, we reported the number of days for which newts kept their PIT-tags: 44.02 days in average; range min-max: 39-69 days. We also noted that none of the tags moved from the insertion site. Newts with transmitters were then radio-tracked up to 48 days after release (see Trochet et al., 2017). Only one newt (ID number: 3910145) was found dead in the field during the survey (seven days after release).

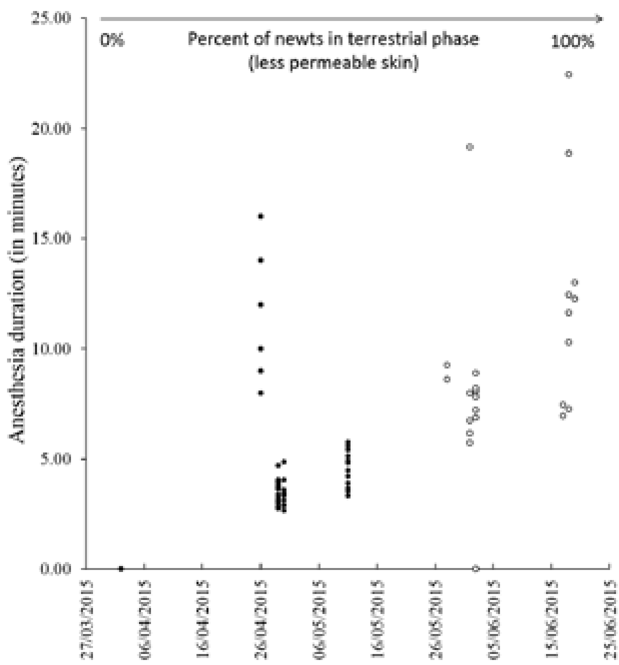

Fig. 3. Anesthesia duration (in minutes) depending on procedure date related to the number of newts in terrestrial phase (less permeable skin). PIT-tagging procedures are represented by black dots and transmitters implantations are represented by white dots.

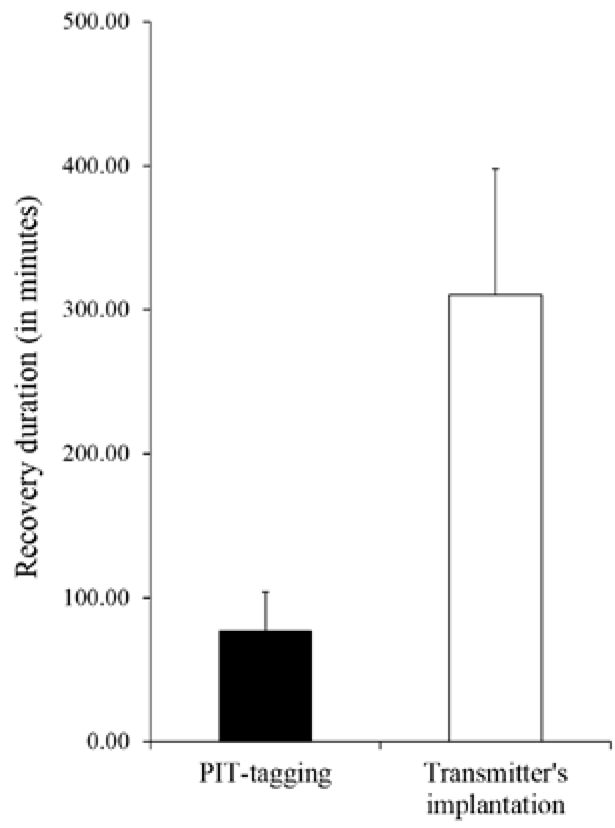

Fig. 4. Recovery duration (in minutes) for both procedures.

DISCUSSION

Marking amphibians is a challenge for studying population dynamics. Indeed, their small size and their semi-aquatic life cycle increase the difficulty to mark them. Toe clipping is still in use in amphibian monitoring, even if this process is actually criticized (Parris and McCarthy, 2001). As a non-invasive and successful method for individual identification, photo-matching is becoming a powerful technique largely used in amphibian species since many years. But this method can be time-consuming, even using automatic processes for photographic re-identification. Moreover, pattern mapping may be not appropriate for studies on large populations (but see Drechsler et al., 2015; Mettouris et al., 2016). PIT-tagging is a recent advance in animal marking technique and is now frequently used in a variety of species, including amphibians (Ott and Scott, 1999; Cucherousset et al., 2008) but this method is relatively costly and had demonstrated contrasting results.

The recent cost reduction and miniaturization of PIT-tags (in this study: PIT-tag length 1.4×8 mm, approximately $2.50 each) make this technique particularly interesting in amphibian monitoring surveys. While photo-identification has been explored in several amphibian species, a detailed and powerful method of PIT-tagging still remains rare, especially in newts. Here, we specified how perform PIT-tagging in the marbled newt to limit PIT-tag loss respecting the welfare of individuals. Also, this kind of marking became mandatory in French establishments authorized to host captive wildlife, and for which a clear protocol is needed.

Anesthesia and recovery durations

The use of a cutaneous anesthetic agent permits the performance of reliable procedures in a less invasive way. Physiological state should be closely observed during the procedures and dosage recommendations followed. Our findings also demonstrated that the recovery duration in newts after anesthesia was more influenced by the invasiveness of the procedure than by the phase of the individuals. We reported deaths after both marking procedures only for two newts operated on twice after the use of surgical adhesive (see below). Hence, we recommend following these protocols to increase the likely success of surveys, even with the constraint of requiring sterile conditions.

PIT-tagging as a robust method for amphibian marking

PIT-tag marking has been used in many species, allowing assessment of movement patterns or growth and survival rates (Jehle and Hödl, 1998; Ott and Scott, 1999; Perret and Joly, 2002). This method is often preferred in long-term population surveys (Perret and Joly, 2002; Arntzen et al., 2004) and in behavioral studies (Winandy and Denoël, 2011), even in relatively small-bodied salamanders (Connette and Semlitsch, 2011). Indeed, no significant effect of PIT-tagging on growth and survival has been demonstrated (Ott and Scott, 1999; Perret and Joly, 2002; Connette and Semlitsch, 2011). PIT-tags are permanent and internal transponders, and this technique is generally considered as less invasive than other methods such as toe clipping which might be a stressful marking method for individuals (Parris and McCarthy, 2001). Most studies using PIT-tagging reported PIT-tag loss and failure, or mortality of individuals, but these results were likely caused by improper implantation (Gibbons and Andrews, 2004). Tag loss can also occur just after PIT-tag injection if the tag exits through the opening caused by the needle (12.1% of tag loss in Feldheim et al., 2002). Most of these studies, which inject PIT-tags without anesthesia, tended to reject the use of these transponders because of low tag retention rate (33.3% tag loss in Brannelly et al., 2014). Here, no PIT-tag expulsion was recorded. Hence, in order to minimize PIT-tag loss, we strongly recommend anesthetizing individuals before PIT-tagging. This results in the immobilization of the individual for several minutes which reduces the chance of expulsion and stress to the individual thereby improving healing. We note that animals can be released just after recovery (i.e., 70.08 ± 20.23 minutes) and kept less than one day in captivity. Using the procedure described here, all newts kept their PIT-tags, even after 69 days. We also applied this protocol previously to *Bufo bufo* (data not shown here) and individuals kept their PIT-tags over 183 days, suggesting that PIT-tags had not been lost by individuals for a long time, and that long-term survey studies should be successful using the protocol described here.

Safe protocol for transmitter implantation for radio-tracking surveys

Most radio-tracking studies in amphibians showed no significant effect on feeding, body mass or survival rate in implanted individuals (Olders et al., 1985; Madison and Farrand, 1998). In amphibians, the use of internal implants is highly recommended, as external tags may hinder mobility and feeding, despite the use of transmitters with various modes of attachment (Fukuyama et al., 1988; Fiorito et al., 1994; Golay, 1996; Tramontano, 1997). Distances and data obtained by radio-tracking are very useful and can be used both in population dynamics and conservation biology studies. To this day, radio-tracking is the only technique that can be used to determine habitat use during seasons where cryptic behavior is displayed, as well as providing indispensable data for conservation management.

Using our protocol of implantable transmitters, we observed no infection after operating on newts. An important observation was made concerning the use of surgical adhesive (3M VETBOND Tissue Adhesive, 3M Animal Care Products, United States) for the 10 first operations, which was applied to the sutures at the end of these procedures. Surgical adhesive may be placed over a suture line for added protection from dehiscence and to serve as a waterproof coating (Wright and Whitaker, 2001). It was observed that these newts had ruptured their sutures due to the solidified surgical adhesive. Newts that had ruptured their sutures were then operated on a second time. Two marbled newts died after this second operation (ID numbers 3910360 and 3560259; Table 1). We believe that these deaths would not have occurred if we not used the surgical adhesive and been obliged to perform a second procedure. As a consequence, the use of surgical adhesive for this particular protocol of transmitter implantation is ill-advised. To summarized, 87.5% animals survived up to 48 days in the wild for successful radio-tracking surveys (see Trochet et al., 2017) post-surgery, until the battery life expired. Transmitter implantation seemed to not impact behaviour of newts, as already observed in other amphibian species (Olders et al., 1985; Madison and Farrand, 1998; Johnson, 2006; Marcec et al., 2016).

Radio-tracking studies however have their limitations: the precision of tracking depends on the number of location points and the presence of an observer may influence the behaviour of the studied animal, even if this last effect is reduced (Brown et al. 2013). Moreover, in small-bodied animals such as newts, the main limitation of radio-tracking studies using implantable transmitters is the transmitter size and battery duration, leading to a restricted signal range (Gourret et al., 2011). Despite the reduction of transmitter size and increased battery life, this restriction could become problematic with very mobile species, for example during migration (van Gelder et al., 1986; Sinsch, 1990).

CONCLUSION

We demonstrated that, in disinfected conditions and following an anesthesia protocol before marking, PIT-tagging can be a very useful marking method, without any tag loss or significant impact on individual health and behavior. Surgical procedures, such as internal transmitter implantation could also be performed under the same anesthesia protocol. Our findings also showed that anesthesia duration was strongly dependent on the phase of newts (aquatic or terrestrial), which is related to certain modifications of the skin, such as permeability. Contrary to the anesthesia duration, the recovery duration was only related to the procedure used (PIT-tagging vs. transmitter implantation).

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Authors have complied with all applicable Animal Care guidelines, and all required French permits (capture permit and authorization for experimentation with animals) have been obtained. AT and HLC collected newts (permit nos. 09-2014-14 and 32-2014-07). DP and SG developed the transmitter implantation protocol in the marbled newt. OC performed surgery (animal experimentation accreditation n°A09-1). HLC and AT analyzed the data and wrote the manuscript, which was corrected by all authors.

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