Ecological Indicators 145 (2022) 109690

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Review

Evaluation of passive integrated transponder tags for marking urodeles

Maria P. Ribas [a,*], Paula Alonso-Almorox [a], Johan Espunyes [a], Albert Martínez-Silvestre [b], Oscar Cabezón [a,c]

[a] Wildlife Conservation Medicine Research Group (WildCoM), Departament de Medicina i Cirurgia Animals. Facultat de Veterinària, Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra 08193, Catalonia, Spain

[b] Catalonia Reptiles and Amphibians Rescue Center (CRARC), Masquefa 08783, Catalonia, Spain

[c] Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra 08193, Catalonia, Spain

ARTICLE INFO

Keywords:
Amphibian conservation
Individual marking
Newts
PIT tag
Population monitoring
Salamanders

ABSTRACT

The use of passive integrated transponder (PIT) tags in urodeles has become popular for individual marking in population and disease ecology studies. However, mark loss or mark-induced mortality can introduce biases and decrease precision in parameter estimates, leading to ineffective population management strategies. In this study we aimed to 1) analyze the existing literature on the use of PIT tags in urodeles; 2) determine whether species characteristics and PIT tagging methods influenced PIT tag rejection across studies; and 3) experimentally assess the adequacy of a subcutaneous PIT tagging method without anesthesia in three European urodele species. We systematically and quantitatively reviewed a database of literature related to the use of PIT tags in urodeles, classified and examined urodele species details, study design, PIT tagging methods, and outcomes across studies. Among the 51 peer-reviewed papers that fit our criteria, the most striking finding was the lack of reporting and standardization of the PIT tagging procedures. The majority of studies presented incomplete information on factors that could strongly influence the probability of PIT tag rejection as well as impact individual welfare (i.e. PIT tag size, its anatomical placement in the animal, anesthesia use, sterility or skin closure methods). We could not identify significant predictors of PIT tag loss, suggesting that the effectivity of PIT tags may be highly specific to the species and method used. Our PIT tagging method proved reliable in *Salamandra salamandra* and *Pleurodeles waltl*, whereas it did not seem a suitable technique for *Calotriton asper* (PIT tag loss was 0% and 66.6%, respectively, and significantly different among species). Overall, we recommend a greater emphasis on reporting implantation methods, ensuring animal welfare and performing species and protocol specific laboratory trials before using PIT tags in urodeles in the field. Critically analyzing PIT tagging methods as well as testing their use in different species is essential to ensure the validity of future research studies and conservation strategies in urodeles.

1. Background

Amphibians are currently the most threatened vertebrate taxa on Earth, with over 41 % of species threatened with extinction and declining more rapidly than either birds or mammals (IUCN, 2021; Stuart et al., 2004). Moreover, the conservation status of amphibians is certainly underestimated since 16.4 % of species are too poorly known to assess (classified as "Data Deficient" by the IUCN). Within amphibian orders, urodeles may be facing greater conservation challenges, reaching 400 out of 701 (57 %) species under threatened categories. The global decline of amphibian populations can be attributed to diverse interacting processes including habitat loss and degradation, overexploitation, invasive species and infectious diseases such as chytridiomycosis (Beebee and Griffiths, 2005; Scheele et al., 2019; Stuart et al., 2004). In order to effectively manage and conserve amphibian diversity, our ability to monitor population dynamics and the impact of specific threats is crucial (Grant et al., 2016; Pickett et al., 2014).

Capture-mark-recapture (CMR) studies are key tools for population ecology, providing estimates of survival, recruitment, abundance and movement (Amstrup et al., 2010; Nichols, 1992; Pradel, 1996). Relevant

* Corresponding author at: Wildlife Conservation Medicine Research Group (WildCoM), Departament de Medicina i Cirurgia Animals. Facultat de Veterinària, Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra 08193, Catalonia, Spain.

E-mail address: mariapuigribas@gmail.com (M.P. Ribas).

https://doi.org/10.1016/j.ecolind.2022.109690
Received 15 August 2022; Received in revised form 9 November 2022; Accepted 14 November 2022
Available online 15 November 2022

aspects of disease ecology and the impact of disease on free-living populations can also be investigated through CMR data (Murray et al., 2009; Wobeser, 2007). All CMR models operate under several assumptions, a critical one being that marks are not lost and do not affect individual survival. Mark loss or mark-induced mortality can introduce biases and decrease precision in parameter estimates, leading to ineffective population management strategies (Arnason and Mills, 1981; McDonald et al., 2003). The choice of a marking technique that has proven reliable for the species under study is clearly important to avoid this issue.

Numerous marking techniques have been used in amphibians including toe clipping, branding and tattooing, subcutaneous injection of fluorescent dyes, Passive Integrated Transponder (PIT) tags and pattern mapping (Ferner, 2010). All of the above-mentioned methods have been used in urodeles, however, each one has apparent advantages and pitfalls. Toe clipping has been a common method for marking urodeles due to its simplicity and low cost. However, mutilation techniques provoke pain and distress and may impact the survival and behavior of marked individuals, raising animal welfare and ethical concerns (McCarthy et al., 2009; Waddle et al., 2008). The potential of toe regeneration, common in urodeles, is another disadvantage of toe clipping and this technique may be inappropriate for long-term studies. Mark durability and readability can also be an issue with fluorescent dye injection and pattern identification (Bailey, 2004; Dalibard et al., 2021; Heemeyer et al., 2007; Moon et al., 2022). Pattern mapping holds promise as a non-invasive and cheap technique, but it can only be used for species with natural patterns or marks and it can be time-consuming in large populations and long-term studies (Arntzen et al., 2004). Nevertheless, constant computational advances are steadily overcoming these issues, simplifying the use of pattern mapping for wildlife studies (Sannolo et al., 2016). At the same time, the use of PIT tags for marking urodeles has become more popular over recent decades, especially as technological advances allow for smaller tags (Cooke et al., 2013; Moon et al., 2022).

Passive Integrated Transponder (PIT) tags are glass-encapsulated circuit chips that, once scanned, provide a unique alpha-numeric code which offer permanent and unambiguous identification (Gibbons and Andrews, 2004). These characteristics satisfy many criteria for an ideal marking technique, such as providing individual identification, potentially lasting indefinitely and being easily read (Ferner, 2010). Tags as small as 7 mm long are currently available, which allows for adaptability to different animal sizes, yet, tag size is still a limitation for its use in smaller urodele species. Tagging can be performed via injection with a pre-loaded needle or by surgical implantation either under the skin, into the muscle or the celomic cavity. Despite being a relatively simple procedure, tagging can involve tissue and organ damage, potentially affecting behavior and even survival of marked individuals. Anesthesia may be required (e.g. depending on the site of tag implantation) and personnel expertise as well as validation of tagging protocols are highly recommended to ensure animal welfare and tag retention (Cooke et al., 2013). As a consequence, PIT tags still present challenges for their applicability in urodeles and in a wide range of situations.

The effectiveness of different PIT tagging techniques has been reviewed for other aquatic taxa such as fishes (Cooke et al., 2011; Ebner, 2009; Musselman et al., 2017) and reptiles (Doody et al., 2009), and there is a large body of literature, dating back to the 1980s, describing its use and implantation in anuran amphibians (Brown, 1997; Camper and Dixon, 1988; Christy, 1996; Pyke, 2005). Conversely, studies assessing the use of PIT tags in urodeles are limited, detailed protocols are rare and data appear scattered. Summarizing PIT tagging techniques and outcomes as well as testing their use in different species is, therefore, essential to ensure the validity of future research studies and conservation strategies in urodeles. In this study we aimed to 1) systematically review the existing literature on the use of PIT tags in urodeles in order to evaluate gaps of knowledge and inform future research; 2) determine whether a relationship between species characteristics and PIT tagging methods on tag rejection was apparent across studies; 3) experimentally assess the adequacy of a subcutaneous PIT tagging method without anesthesia in three European urodele species.

2. Methods

2.1. Literature review

We conducted a systematic quantitative literature review, in order to provide a comprehensive and reproducible overview of the current knowledge on the use of PIT tags in urodeles (Pickering and Byrne, 2014). We used *Scopus* (Elsevier) and *Web of Science* (WoS; Thomson Reuters) databases using 'title, abstract, keyword' and 'all fields' searches respectively. We used the keywords "(urodel* OR caudat* OR salamand* OR newt) AND (PIT-tag* OR transponder* OR microchip*)" (29 articles from *Scopus*, 35 articles from *WoS*). We also conducted a search on Google Scholar and the reference list of extracted articles was screened to retrieve additional relevant literature.

We then assessed the title and abstract of each entry individually for inclusion in our database. Criteria for inclusion were peer-reviewed primary literature and English-language full text; whereas exclusion criteria were study/paper duplicates, non-peer reviewed/grey literature (i.e., books, workshops, theses, governmental reports), studies reporting the use of implantable marks/tracking devices other than PIT tags (e.g. radio-transmitters) and studies with a single time point, where study design precluded information about PIT tag effectivity. The full-text of the remaining articles was assessed in order to further filter the results. For each paper that met our inclusion/exclusion criteria we thoroughly reviewed their content and extracted the following data to construct a database: literature details, urodele species details, study details, PIT tagging methods and outcomes.

Under 'urodele species details' we recorded taxonomic family, species identity and IUCN status (IUCN, 2021) and population origin. We also added the preferred habitat and mean total length of the species in our database (Oliveira et al., 2017). For 'study details' we extracted study focus, sample size, location (i.e., laboratory, mesocosm or field study) and duration of the study. In study focus, we classified studies according to their main objectives in 1) method validation, when aiming to estimate PIT tag effectivity, health impacts or compare it with other marking methods; 2) population level, when PIT tags were used to estimate population parameters; 3) animal movement, when PIT tags were used to evaluate activity patterns, habitat preferences or dispersal; and 4) others, when PIT tags were used as a reference mark for other studies. 'PIT tagging methods' included PIT tag size, tag location, use of anesthesia, skin closure and sterility assurance. Under 'outcomes' we recorded or calculated information on survival (proportion of animals alive at the end of the experiment), tag rejection (proportion of animals that expelled PIT tags) and recapture (proportion of animals recaptured at least one time at the end of the experiment).

Data was extracted from all publications included in the systematic database independently by two investigators and then compared for consistency. For our analysis we split studies into experiments if they used different species, methodologies, times or study locations. If retention, survival or recapture were reported at multiple times through an experiment, we used the information from the last time step. Finally, we summarized and analyzed the resulting database to detect patterns.

2.2. Data analysis

We used the extracted data to test whether a relationship between PIT tag rejection and species characteristics and tagging methods was apparent. For the *meta*-analysis, we used the studies which reported PIT tag rejection data and that had complete records on all other variables of interest. The potential predictors of PIT tag rejection were selected based on similar studies in other taxa (Musselman et al., 2017) and on information availability. We constructed a generalized linear model with PIT tag rejection as response variable and taxonomic family, habitat preferences, tagging anatomical location and the ratio of tag length to animal length (tag:urodele length) as predictors. Animal length rather than mass was used since length measurements are more commonly reported in PIT tagging studies.

2.3. Experimental PIT tagging

We conducted laboratory experiments on three species of European urodeles to evaluate the validity of a subcutaneous PIT tagging method without anesthesia. The species included in the study were fire salamander (*Salamandra salamandra,* n = 18), Pyrenean brook newt (*Calotriton asper*, n = 9) and sharp-ribbed newt (*Pleurodeles waltl*, n = 12). The individuals used in this experiment were confiscated from illegal collectors and were admitted at the Catalonian Reptiles and Amphibians Rescue Center (Barcelona, Spain). They were housed in groups of 5–10 individuals of the same species in 600 × 400 × 300 mm aquaria (room temperature 20 °C, water temperature 14–18 °C, water pH 7, vegetable soil for fire salamanders and water and pebbles for the newts). Water was regularly changed and aerated by aeration stones, and the daylight/dark pattern was set at 14/10 h according to the local natural photoperiod. All the individuals included in the study were apparently-healthy, chytrid fungi and ranavirus-negative adults that had been acclimated to captive conditions for a minimum of one month.

In 2020, we implanted PIT tags (ID-100A, 8 × 1.4 mm, Trovan ltd.) subcutaneously in the dorsum of each individual following a previously described protocol for anurans (Christy, 1996; Newell et al. 2013). This method does not require anesthesia and has shown no effects on behavior, appetite, mobility or cause of morbidity/mortality of marked anurans (Christy, 1996). Transponders were supplied in individually-packed sterilized needles and were applied using a spring-loaded plastic syringe. The needle was inserted subcutaneously in the dorsum of the animal, parallel to the spine, at the level of the caudal thorax and facing cranially. At a distance of 1–2 cm from the entrance wound, the tag was injected by applying pressure to the syringe plunger. This tag placement ensured that internal organs were not damaged since they were protected by the rib cage. The site of injection was sealed with Vetbond® tissue adhesive and the animals were then returned to their aquaria. The animals were followed up at 2, 7, 14, 21 and 28 days after PIT tag placement. At each follow-up, we recorded survival and we inspected each individual for tag loss and stage of wound healing. Wounds were considered in an inflammatory stage when marked inflammation was observed; in a proliferative stage when granulation tissue was present and wound edges began to contract; and in a maturation stage when scar tissue formed (Poll, 2009; Young and McNaught, 2011).

Tagging was performed by a trained veterinary surgeon (M.P.R.) using a pair of new and previously-moistened nitrile gloves. Survival (percentage alive at the end of the trial), tag retention (percentage retaining tags at the end of the trial), and time to healing were calculated at the conclusion of the experiment. Kaplan-Meier survival curves were constructed for each species using PIT tag rejection as the response variable, and differences among species were tested using a log-rank test. All statistical analyses were performed with R 4.0.3 (R Core Team, 2022) and using “ggplot2” version 3.3.6 (Wickham, 2016) and “survival” version 3.3-1 (Therneau, 2022) packages.

3. Results

3.1. Literature review

The literature search returned 29 articles identified by *Scopus*, 35 articles identified by *WoS* and 37 articles identified by Google Scholar and paper citations, resulting in 76 research papers after removing duplicates. Seven articles were excluded based on title and abstract and 18 additional articles were excluded after full-text screening. Overall, we obtained 51 original peer-reviewed research papers that met our criteria of PIT tag studies on urodeles (Appendix S1 for complete list of studies reviewed and extracted data). Publication dates ranged from 1993 to 2022, with an increase in the number of published papers since 2006 and a peak in publications in 2017 (n = 7). Papers were published in 29 different journals and the three journals publishing the highest number of papers were *Herpetological Review* (n = 8), *Herpetological Journal* (n = 5) and *Journal of Herpetology* (n = 5). The majority of studies were conducted in United States (58.86 %; 29/51). Six studies were carried out in France; three each in Germany and Austria; one each in Belgium, Canada, Czech Republic, Italy, Japan, Laos, Mongolia, Spain and UK; and one study was carried out in both France and Spain. Regarding the focus of the studies, 19 were centered on method validation, 15 on population level estimations, nine on animal movement, and eight studies used PIT tags as a reference mark for other studies (Fig. 1).

Within the 51 retrieved studies we could identify 65 individual experiments. These experiments were conducted on 29 species of urodeles, with 12 species used in multiple experiments (Table 1). These species corresponded to seven families: Salamandridae (n = 10), Ambystomatidae (n = 7), Plethodontidae (n = 6), Sirenidae (n = 2), Hynobiidae (n = 2), Cryptobranchidae (n = 1), Proteidae (n = 1). Most papers focused on a single urodele species (82.35 %; 42/51) and eight studies used two species. A single study contained information on three species (*Ambystoma annulatum, A. maculatum* and *A. texanum*), however, the authors reported study outcomes together for the three species and we considered them as a single experiment (i.e., *Ambystoma spp*. on Table 1). The adult mean length of the studied species ranged from 87.5 mm of *Ichthyosaura alpestris* to 978 mm of *Siren lacertina* (median = 164.25).

Experiments were disproportionally conducted on species classified as ‘least concern’ (52/65), followed by ‘near threatened’ (10/65), and only three of the 65 experiments were performed on ‘vulnerable’ species (IUCN, 2021; Fig. 2a). No experiments included species under other threatened categories. Species with terrestrial habits were more commonly used in experiments (42/65) than either species with aquatic habits (14/65) or shared terrestrial-aquatic preferences (9/65) (Fig. 2b). Experiments were also more frequently conducted on adult life-stages (55/65), with only eight experiments conducted on juveniles and one each on metamorphs and larvae (Fig. 2c). Experiments also varied in the location where they were conducted, the majority being performed in the field (40/65), but laboratory settings (19/65) and mesocosms (6/65) were also used (Fig. 2d). There were three articles that included both laboratory and field setups and one that combined laboratory and mesocosm. The sample size (number of PIT tagged urodeles) per experiment ranged from 6 to 3,745 individuals (median = 79) and it was higher in field studies (median = 147) than in laboratory and mesocosm settings (median = 18). Experiment duration was highly variable, ranging from 7 days to 20 years (median = 377.5 days) and it was also greater for field (median = 730 days) than for laboratory and mesocosm settings (median = 106.5 days). Sample size and experiment duration were not specified in three experiments each.

Regarding PIT tagging methods, the most common implanting location was the celomic cavity (30/65), followed by subcutaneous (20/65) and into the tail musculature (8/65) (Fig. 2e). Subcutaneous PIT tags were either inserted dorsally (8 experiments) or ventrally (12 experiments). The location of tag implantation was not mentioned in seven experiments. Additionally, we were uncertain about the true location of tags in two studies classified as subcutaneous, but where the authors later mentioned ambiguous sentences, such as referring to tags into the “body cavity”. PIT tags were most frequently implanted under anesthesia (40/65) whereas anesthesia was either not used or not mentioned for the rest of experiments (25/65) (Fig. 2f). Anesthesia methods included the topical use of the following agents: tricaine methanesulfonate (MS222; 24 experiments), 2-phenoxyethanol (6 experiments), Orajel® (20 % benzocaine; 4 experiments), chloretone in water (3 experiments), EMLA® cream (2.5 % lidocaine and 2.5 % prilocaine; 2 experiments) and clove oil (1 experiment). PIT tags were implanted in all body locations irrespective of the use of anesthesia, but anesthesia

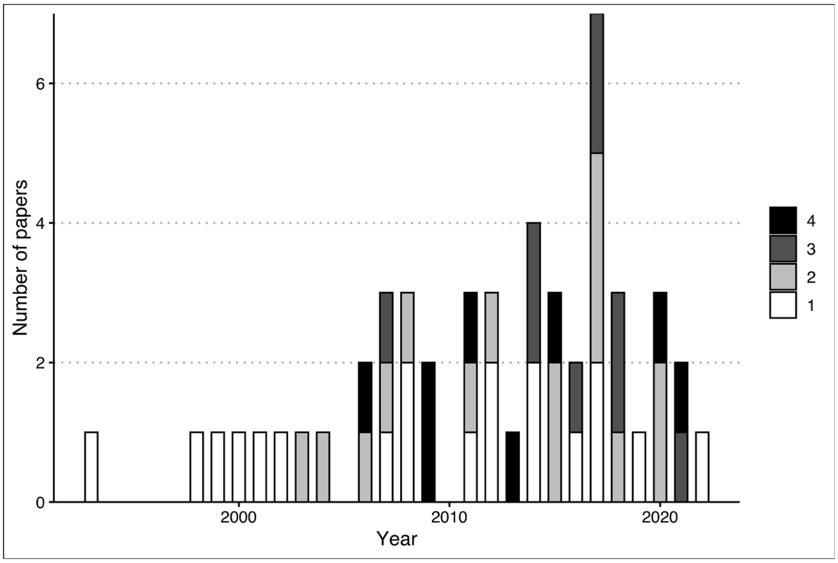

Fig. 1. Bar graph showing the number of papers published in each of the study foci by publication year. 1: method validation; 2: population level; 3: animal movement; 4: other study focus.

Table 1
Taxonomic families and species of urodeles on which the 65 reviewed experiments were conducted.

Family species	N° experiments
Ambystomatidae	**23**
Ambystoma annulatum	5
Ambystoma laterale	4
Ambystoma macrodactylum	1
Ambystoma maculatum	7
Ambystoma opacum	3
Ambystoma tigrinum	2
Ambystoma spp	1
Cryptobranchidae	**5**
Cryptobranchus alleganiensis	5
Hynobiidae	**2**
Hynobius nebulosus	1
Salamandrella keyserlingii	1
Plethodontidae	**8**
Desmognathus monticola	2
Desmognathus quadramaculatus	1
Eurycea rathbuni	1
Plethodon albagula	1
Plethodon metcalfi	1
Plethodon shermani	2
Proteidae	**1**
Necturus maculosus	1
Salamandridae	**24**
Calotriton asper	3
Ichthyosaura alpestris	8
Pleurodeles waltl	1
Salamandra salamandra	4
Taricha torosa	1
Triturus carnifex	1
Triturus cristatus	3
Triturus dobrogicus	1
Triturus marmoratus	1
Tylototriton podichthys	1
Sirenidae	**2**
Siren intermedia	1
Siren lacertina	1
Total	**65**

was predominantly used in intracelomic and ventral subcutaneous implants (Fig. 3).

Experiments used diverse PIT tag sizes, with 12–12.5 mm tags being more frequently used (17/65), followed by 8–8.5 mm (10/65), 11–11.5 mm (7/65), 9–9.5 mm and 13 mm tags (1/65 each) (Fig. 2g). Numerous experiments did not mention tag sizes (23/65), accounting for 35.38 % of all experiments. Six experiments used various tag sizes depending on the length of the individual, however, the number of animals marked with each tag size was not specified and results were combined for all tag sizes. The ratio of tag length to animal length (tag:urodele length) could be calculated in 33 experiments, ranging from 0.018 to 0.137. Methods of skin closure after PIT tagging were not commonly used or reported (53/65). Eleven experiments mentioned the use of tissue adhesive and a single experiment used traditional sutures for wound closure, but they were removed three days after tagging (Fig. 2h). We could only identify-five experiments where sterility was ensured throughout the PIT tagging procedure, in which tags were injected with sterile needles in four cases while sterile tags were surgically inserted with a sterile technique in one experiment. The remaining experiments were divided between injected tags where sterility was not mentioned (24/65), and surgically inserted tags where sterility of all materials (e.g., PIT tags, scalpel blades, etc.) was not ensured or where these had been rinsed with disinfectants (21/65). We were not able to determine sterility conditions for 15 experiments. Injection with a needle was the only method used to implant PIT tags into the tail musculature, whereas both injection and surgical insertion were used for all other anatomical locations.

Information on tag rejection was only available for 29 experiments and ranged from 0 to 45.45 % (mean = 5.3 %, median = 0 %). No tag rejection was detected in 29.23 % of experiments (19/65). Survival of urodeles in laboratory and mesocosm setups ranged from 44.4 to 100 % (mean = 95.58 %), with most experiments reporting 100 % survival (15/25) and four mesocosm experiments lacking information about survival. In field studies, survival could only be calculated in one experiment where all individuals were recaptured alive after PIT tagging. Nevertheless, recapture rates were available for 29 field experiments, ranging from 3 to 100 % (mean = 36.48 %, median = 30 %). Additionally, two out of the four mesocosm studies that did not report survival described recapture rates of 49 % and 80 %, which could be interpreted as a proxy for survival.

3.2. Data analysis

The final model for the predictors of PIT tag rejection was based on 21 experiments from 16 urodele species within 15 unique publications. None of the hypothesized factors were significant predictors of PIT tag rejection ($p > 0.05$).

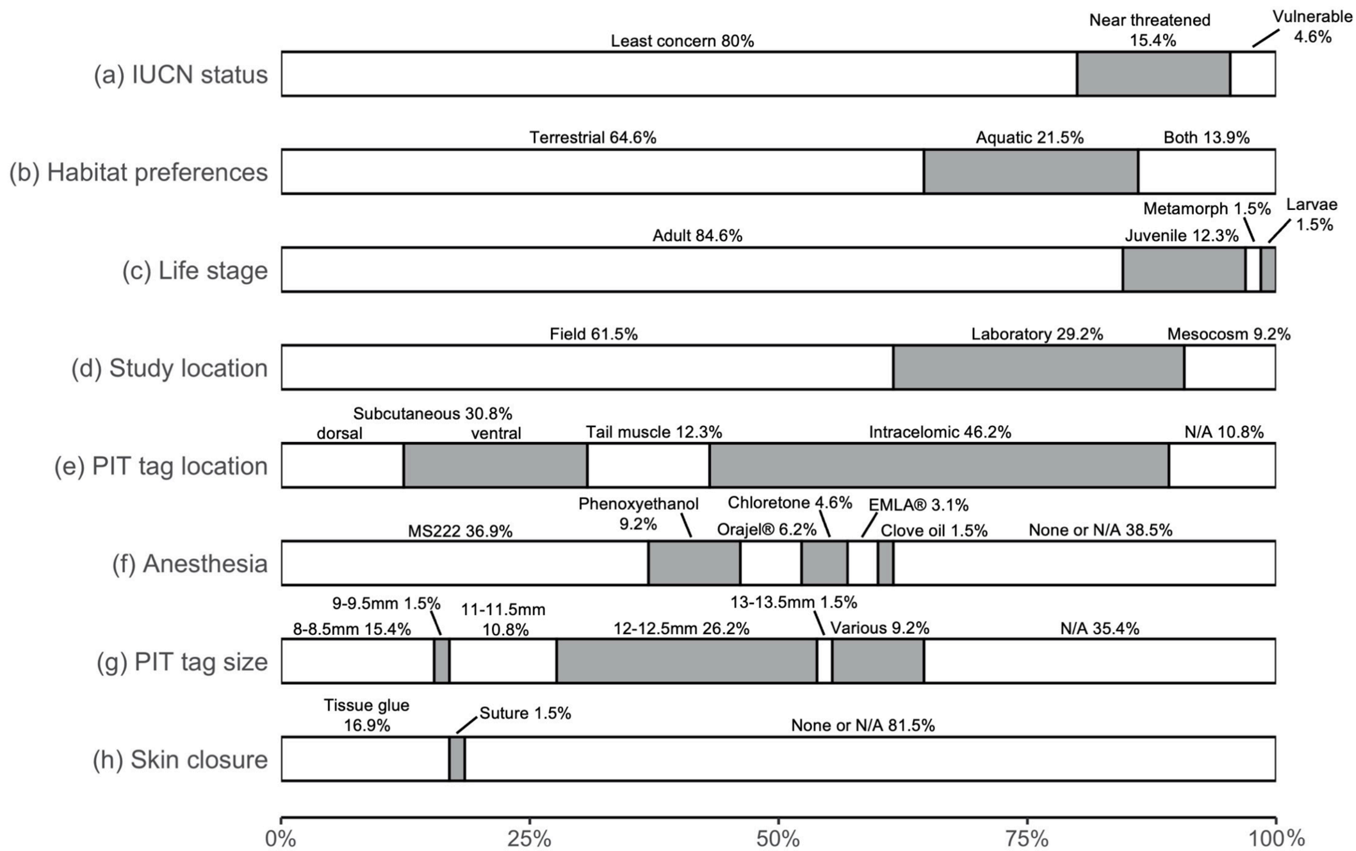

Fig. 2. Bar graph demonstrating the proportion of (a) species grouped according to IUCN status, (b) species grouped according to habitat preferences, (c) urodele life stages, (d) Study location, (e) anatomical location of PIT tag implants, (f) anesthetic methods, (g) PIT tag size, and (h) methods of skin closure, used on the 65 experiments reviewed. N/A: not available.

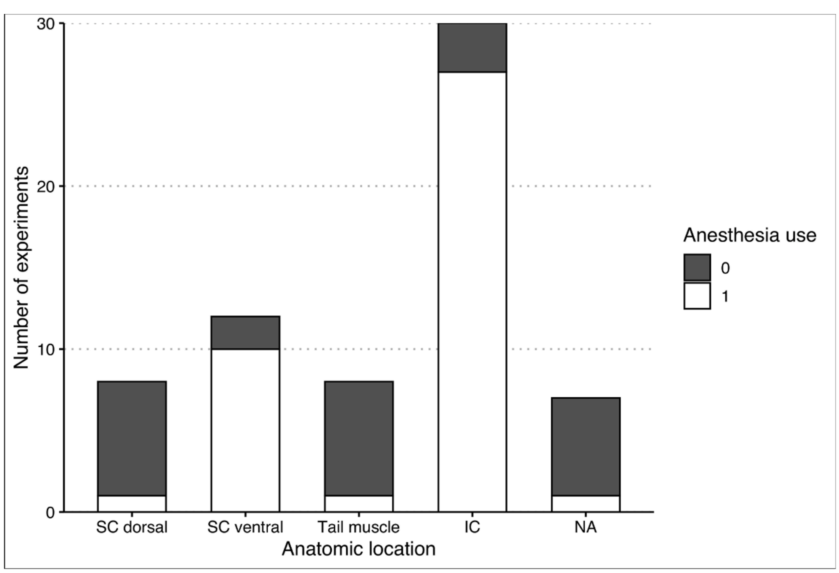

Fig. 3. Bar graph showing the number of experiments that used anesthesia in each anatomical location where passive integrated transponders were implanted in urodeles. SC: subcutaneous; IC: intracelomic; NA: not available; 0: anesthesia was not used or not mentioned; 1: anesthesia was used during the tagging procedure.

3.3. Experimental PIT tagging

Survival was 100 % for all species throughout the experiment. PIT tag retention was 100 % in fire salamanders and sharp ribbed newts. Wounds in fire salamanders progressed to a proliferative stage 7 days post-tagging, were remodeling 14 days post-tagging and were considered mature 21 days after tagging. In sharp-ribbed newts, wounds also reached a proliferative stage 7 days post-tagging but this stage extended until the next control, 14 days after tagging. At this time point, two individuals showed wounds coated with a whitish filamentous material, compatible with fungal hyphae. In the following control (21 days post-tagging), four animals were considered to be maturing whereas two individuals continued on a proliferative stage and showed crusty lesions. The two sharp-ribbed newts that showed fungal-like growth on the past control, presented large wounds in an inflammatory stage, suggesting a step back in the healing process probably due to wound infection. On the last control, 28 days post-tagging, these two individuals were on an advanced proliferative stage while the rest of sharp-ribbed newts had mature wounds.

In Pyrenean brook newts, overall PIT tag retention was 33.3 %. Three animals lost tags after 7 days and there was one additional tag loss each at 14, 21 and 28 days. PIT tag retention was significantly different between Pyrenean brook newts and the other species (Fig. 4). At day 7 post-tagging, all newts were considered to be in an inflammatory stage of wound healing and presented larger wounds than the other species. After 14 days, wounds appeared to be in a proliferative stage in four of the remaining tagged Pyrenean brook newts while one showed marked inflammation and a cystic lesion filled with whitish material, suggestive of infection and/or necrosis. Interestingly, wound maturation had already started in the Pyrenean brook newts that had lost their tags. In the following control (21 days post-tagging), the individual that presented wound infection had lost its tag and another animal still presented a proliferative wound. The rest of animals presented mature wounds. On the final control (28 days post-tagging), the animal with proliferative wounds had lost its tag while the remaining tagged Pyrenean brook newts had mature wounds. Tissue adhesive remained attached in most individuals from the three species for up to 14 days.

4. Discussion

The findings of our study evaluated the extent of use and effectivity of PIT tags for marking urodeles, including study characteristics, urodele species, PIT tagging methods and overall findings. Moreover, our work allowed testing potential predictors of PIT tag rejection and offered a PIT tagging method that proved reliable and ready-to-use for *S. Salamandra* and *P. waltl*. Here we critically analyze our findings in order to assist population monitoring programs and to improve individual welfare.

4.1. Limited validation studies and potential biases in population studies

The results of our systematic review provide a comprehensive picture of the use of PIT tags in urodeles over a 30-year period. In the past decades, the recognition of global amphibian declines and the emergence of devastating amphibian diseases have boosted the study of this group of vertebrates (Scheele et al., 2019; Stuart et al., 2004). In parallel, PIT tag technology has increased steadily in amphibian research due to its advantages over traditional marking methods in population monitoring (Gibbons and Andrews, 2004). The general growth of this field of research is reflected in the increase of scientific production identified by our literature review over the past two decades. Despite this development in research, we found limited studies focusing on validation of PIT tagging methods for particular urodele species.

Similar to other marks, PIT tags are not exempt of disadvantages such as tag loss or impacts on individual survival. Without proper validation of specific protocols for PIT tag implantation it is, therefore, possible to obtain inaccurate estimates of population parameters that are relevant for amphibian conservation. Through our systematic search, we could only identify 19 studies where the main focus was to describe and evaluate the performance of PIT tags in urodeles. These results are concerning since they indicate that many population studies are performed without prior knowledge on the limitations of this marking technique. Moreover, while controlled laboratory studies offer the best evidence of PIT tagging performance, they tended to be constrained in sample size and study duration. In our laboratory trial as well as in other works (Moon et al., 2022), PIT tag losses have been documented up to a month after implantation, suggesting that shorter experiment durations may underestimate tag losses. A cautious approach would be to set the end of the experiment when skin wounds

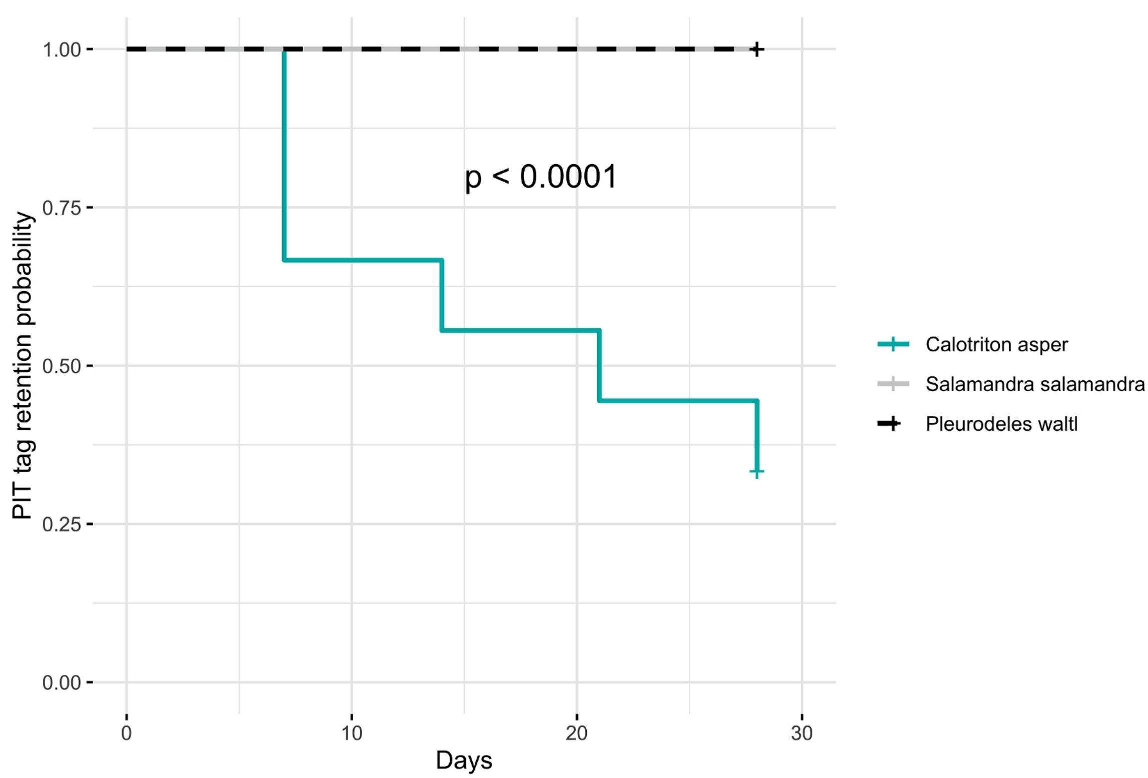

Fig. 4. Kaplan–Meier analysis and survival plot showing PIT tag retention over 28 days in Pyrenean brook newts (*Calotriton asper*, n = 9), fire salamanders (*Salamandra salamandra,* n = 18) and sharp-ribbed newts (*Pleurodeles waltl,* n = 8). Tag retention rates were significantly lower in Pyrenean brook newts compared with the other species ($X^2 = 23.9$; df = 2, $p = 6e^{-06}$).

are completely healed, minimizing the possibility of tag loss. We found that mean PIT tag rejection across studies was relatively low (5.3 %), but some experiments reported alarming rejection rates, reaching 45.5 %. For field experiments, these numbers may be significantly higher since they can only estimate minimum PIT tag losses, when free PIT tags are found in the field – a challenging task.

We acknowledge that significant publications on PIT tag use in urodeles may have been missed by our search due to the inherent limitations of systematic reviews. Grey literature and valid literature produced in languages other than English were excluded by the platforms that we used to search the literature. The specific search terms and search fields used to find publications restricted the retrieval of literature that did not include our terms. Nevertheless, studies focusing on method validation were the most commonly captured by our search and we are thus confident that our results offer a valid representation of the use of PIT tags in urodeles. Overall, our data indicates that current information on urodele population dynamics based on CMR studies using PIT tags could be significantly biased, potentially overestimating population sizes while underestimating the threat status of some species.

4.2. The use of PIT tags was restricted to a small proportion of urodele diversity

There are currently 701 species of extant urodeles described, representing 70 different genera and 9 families (IUCN, 2021). Our review found that PIT tags have been used in almost all families (7/9 families). However, only 17 genera and 29 species were included in the reviewed studies, representing solely 24.3 % and 4.1 % of urodele diversity, respectively. When comparing the range of total length between all extant urodeles (20–1,360 mm) and the species represented by our review (87.5–978 mm), it is also clear that PIT tags have not been used in the entire range of urodele sizes. Moreover, the majority of experiments were performed on adult animals, whereas juvenile and premetamorphic stages were hardly studied. These life stages can be significantly smaller in body size than adults and, therefore, PIT tags may not be suitable marking techniques for them.

Urodeles are distributed throughout most of the Holarctic, with a hotspot of diversity in the United States and few species occurring below the equator in South America. This was consistent with our findings that most studies were conducted in the United States. Nevertheless, it was surprising that many European countries were overlooked in our review as well as the East Asia region, North Africa and South America. These results show an important gap of knowledge on the use of PIT tags in urodele species native to these areas. Of particular concern, only three experiments were conducted on threatened urodele species. Considering that there are 400 threatened urodele species and the potential of PIT tags in assisting population monitoring, it is crucial to validate PIT tagging methods in a greater number of species in order to inform conservation programs.

4.3. PIT tag implantation procedures were imprecise and not standardized

We detected a striking lack of detail in the description of the PIT tagging procedures and methods in the reviewed studies. The majority of studies did not offer complete reports of fundamental details including PIT tag size, its anatomical placement in the animal, the use of anesthesia, level of sterility or the use of skin closure methods. It is, therefore, difficult to draw comparisons and conclusions about the performance of different implantation procedures using this incomplete data. Nevertheless, these factors could strongly influence the probability of PIT tag rejection as well as impact individual welfare as reported for other ectothermic species (Gibbons and Andrews, 2004; Lyon et al., 2019; Musselman et al., 2017).

Inadequate standardization in the use of specific methods during PIT tagging protocols was also concerning. In particular, we detected a great inconsistency in the use of anesthesia and in the selection of anesthetic methods. Anesthesia may be indicated in PIT tagging procedures to achieve adequate muscle relaxation or to reduce pain and discomfort of the animals. Implantation of a PIT tag into the celomic cavity can be considered equivalent to intra-celomic surgery and should involve the use of anesthesia as well as maximum levels of sterility and muscle and skin closure, which were not ensured across the reviewed studies. Additionally, we identified the use of anesthetic methods that may require further evaluation in amphibians since high mortality and inconsistent anesthetic depth have been reported, namely Orajel® and EMLA® cream (Baitchman and Stetter, 2014). On the other hand, may require additional permits, special equipment and trained personnel, which can prevent its use under field conditions. The selection of an acceptable anatomical location for PIT tag placement, among others, could help avoiding the use of anesthesia.

The selection of the size of the PIT tag was also not standardized and inconsistent with the size of the urodele species under study. Currently, there are no evidence-based guidelines for the minimum animal size for PIT tagging and rules-of-thumb such as the “2% rule” for fish (Winter, 1996) have been strongly opposed (Brown et al., 1999; Jepsen et al., 2005). However, it is clearly important to minimize PIT tag size in relation to animal body size in order to reduce the probability of PIT tag rejection and to improve animal welfare (Jepsen et al., 2005). Altogether, PIT tagging is a procedure that entails important risks for the animals and we recommend that it is always conducted under the supervision of a trained veterinary surgeon and following the appropriate regulations of each country.

4.4. PIT tags need to be validated for each particular species and implantation method

Using data from the reviewed studies, we were not able to identify predictors of PIT tag rejection in urodeles. Conversely, studies in fishes have identified several factors that can greatly influence PIT tag retention, such as anatomical location or the use of sutures (Musselman et al., 2017). Due to the inconsistency in reporting the methods for PIT tag implantation, we could only include 21 out of 65 experiments in our final model, which may have influenced our ability to identify significant predictors. These results suggest that the effectivity of PIT tags may be highly specific of the species and method used and further highlight the need for conducting controlled studies before marking free-living populations.

In the present study, we contributed to this need by evaluating the performance of a PIT tagging method without anesthesia in three European species of urodeles. Our protocol presents some advantages for field studies such as that no anesthesia is required. Moreover, we demonstrated that Vetbond® or equivalent tissue adhesives are useful for wound closure, possibly contributing to wound healing and to PIT tag retention in the field. Our results showed significant differences in the effectivity of PIT tags between species. On one hand, PIT tags have been demonstrated as reliable marks in both fire salamanders and sharp-ribbed newts. Conversely, our PIT tagging method is not a suitable technique for marking Pyrenean brook newts, both in terms of reliability and animal welfare.

Lesions in Pyrenean brook newts were considerably larger and took over a month to heal while the PIT tag was still in place. The appearance of lesions and the rapid healing after PIT tag loss, are indicative of a foreign body reaction to the PIT tag (Kastellorizios et al., 2015). Pyrenean brook newts were the smallest species studied (i.e., total length 160 mm compared to >280 mm in the other two species studied), suggesting that body size may be important for PIT tag retention. Despite all PIT tags were retained in sharp-ribbed newts, delayed healing and wound infection in some individuals raise welfare concerns when using our protocol. Sharp-ribbed newts have an average total length of 300 mm and wounds were markedly different from those of Pyrenean brook newts. However, both species were PIT tagged during an aquatic phase and it is plausible that delayed healing is related to the aquatic environment. Further studies should test our PIT tagging method during a terrestrial phase to determine its safety in sharp-ribbed newts.

5. Conclusions

In this quantitative systematic review, we critically analyzed the use of PIT tags in urodeles on a worldwide scale, providing valuable information related to publication numbers, species, methods and reported success of this marking technique, as well as attempted to identify predictors of PIT tag loss. In particular, we identified a striking lack of standardization on reporting the protocols used for PIT tagging these species, together with unpredictable patterns of tag loss. We recommend a greater emphasis on reporting implantation methods, ensuring animal welfare and performing species and protocol specific laboratory trials before using PIT tags in the field. We also described a PIT tagging technique without anesthesia that can be reliable for marking fire salamanders, sharp-ribbed newts, and potentially other urodele species. Urodeles are suffering dramatic declines worldwide and capture-mark-recapture studies with reliable marks are essential tools for population and disease ecology. Optimization of individual marking techniques, such as PIT tags, is therefore crucial for evidence-based management and conservation of endangered urodele species.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

We have shared our data as Supplementary Files

Acknowledgments

We thank Joaquín Soler and Zuleika Alonso from the Catalonia Reptiles and Amphibians Rescue Center (CRARC) and Diego Martínez-Martínez for the care of the animals during the experimental PIT tagging. We also thank J. Bosch and B. Thumsóva (SOS ANFIBIOS) for testing the study animals for common amphibian pathogens. We wish to express our appreciation to Trovan Ltd. for providing technical support. M. P. R. was funded through the 2021 FI Scholarship, Departament de Recerca i Universitats, Generalitat de Catalunya, Spain (FI_B 00171). Animal manipulation and PIT tagging were approved by the competent authorities (Generalitat de Catalunya, Spain, PTOP-2020-293).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.109690.

References

Amstrup, S.C., McDonald, T., Manly, B.F.J., 2010. Handbook of Capture-Recapture Analysis. Princeton University Press.

Arnason, A.N., Mills, K.H., 1981. Bias and loss of precision due to tag loss in Jolly-Seber Estimates for Mark-Recapture experiments. Can. J. Fish. Aquat. Sci. 38, 1077–1095. https://doi.org/10.1139/f81-148.

Arntzen, J.W., Goudie, I.B.J., Halley, J., Jehle, R., et al., 2004. Cost comparison of marking techniques in long-term population studies: PIT-tags versus pattern maps. Amphibia-Reptilia 25, 305–315.

Bailey, L.L., 2004. Evaluating elastomer marking and photo identification methods for terrestrial salamanders: marking effects and observer bias. Herpetol. Rev. 35, 38.

Baitchman, E., Stetter, M., 2014. Amphibians, in: West, G., Heard, D., Caulkett, N. (Eds.), Zoo Animal and Wildlife Immobilization and Anesthesia, 2nd Edition. Wiley-Blackwell, pp. 303–311. https://doi.org/doi:10.1002/9781118792919.ch17.

Beebee, T.J.C., Griffiths, R.A., 2005. The amphibian decline crisis: a watershed for conservation biology? Biol. Conserv. 125, 271–285. https://doi.org/10.1016/j.biocon.2005.04.009.

Brown, L.J., 1997. An evaluation of some marking and trapping techniques currently used in the study of anuran population dynamics. J. Herpetol. 410–419.

Brown, R.S., Cooke, S.J., Anderson, W.G., McKinley, R.S., 1999. Evidence to challenge the "2% rule" for biotelemetry. North Am. J. Fish. Manag. 19, 867–871.

Camper, J.D., Dixon, J.R., 1988. Evaluation of a microchip marking system for amphibians and reptiles. Texas Parks \& Wildlife Department.

Christy, M., 1996. The efficacy of using Passive Integrated Transponder (PIT) tags without anaesthetic in free-living frogs. Aust. Zool. 30, 139–142.

Cooke, S.J., Woodley, C.M., Brad Eppard, M., Brown, R.S., Nielsen, J.L., 2011. Advancing the surgical implantation of electronic tags in fish: a gap analysis and research agenda based on a review of trends in intracoelomic tagging effects studies. Rev. Fish Biol. Fish. 21, 127–151.

Cooke, S.J., Midwood, J.D., Thiem, J.D., Klimley, P., Lucas, M.C., Thorstad, E.B., Eiler, J., Holbrook, C., Ebner, B.C., 2013. Tracking animals in freshwater with electronic tags: past, present and future. Anim. Biotelemetry 1, 1–19.

Dalibard, M., Buisson, L., Calvez, O., Nguyen-hong, M., Trochet, A., Laffaille, P., 2021. Can ventral pattern be used for individual recognition of the vulnerable Pyrenean brook newt (*Calotriton asper*)? Herpetol. J. 31, 99–110.

Doody, J.S., Roe, J., Mayes, P., Ishiyama, L., 2009. Telemetry tagging methods for some freshwater reptiles. Mar. Freshw. Res. 60, 293–298.

Ebner, B.C., 2009. Preface. Tagging for telemetry of freshwater fauna. Mar. Freshw. Res. 60, 281–283.

Ferner, J.W., 2010. Measuring and marking post-metamorphic amphibians. Amphib. Ecol. Conserv. A Handb. Tech. 123–141.

Gibbons, W.J., Andrews, K.M., 2004. PIT Tagging: Simple Technology at Its Best. Bioscience 54, 447–454. https://doi.org/10.1641/0006-3568(2004)054[0447:PTSTAI]2.0.CO;2.

Grant, E.H.C., Miller, D.A.W., Schmidt, B.R., Adams, M.J., Amburgey, S.M., Chambert, T., Cruickshank, S.S., Fisher, R.N., Green, D.M., Hossack, B.R., Johnson, P.T.J., Joseph, M.B., Rittenhouse, T.A.G., Ryan, M.E., Waddle, J.H., Walls, S.C., Bailey, L.L., Fellers, G.M., Gorman, T.A., Ray, A.M., Pilliod, D.S., Price, S. J., Saenz, D., Sadinski, W., Muths, E., 2016. Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. Sci. Rep. 6, 1–9. https://doi.org/10.1038/srep25625.

Heemeyer, J.L., Homyack, J.A., Haas, C.A., 2007. Retention and readability of visible implant elastomer marks in eastern red-backed salamanders (Plethodon cinereus). Herpetol. Rev. 38, 425.

IUCN, 2021. The IUCN Red List of Threatened Species. Version 2021-3. https://www.iucnredlist.org. Accessed on 5 March 2022. [WWW Document].

Jepsen, N., Schreck, C., Clements, S., Thorstad, E.B., 2005. A brief discussion on the 2% tag/bodymass rule of thumb. Aquat. Telem. Adv. Appl. 255–259.

Kastellorizios, M., Tipnis, N., Burgess, D.J., 2015. Foreign body reaction to subcutaneous implants. Immune Responses to Biosurfaces 93–108.

Lyon, J.P., Hale, R., Kitchingman, A., O'Connor, J., Sharley, J., Tonkin, Z., 2019. Effects of tag type, morphological location and tagger experience on tag retention rates in freshwater fishes. Mar. Freshw. Res. 70, 891–895.

McCarthy, M.A., Weller, W.F., Parris, K.M., 2009. Effects of toe clipping on survival, recapture, and return rates of Jefferson Salamanders (Ambystoma jeffersonianum) in Ontario, Canada. J. Herpetol. 43, 394–401.

McDonald, T.L., Amstrup, S.C., Manly, B.F.J., 2003. Tag loss can bias Jolly-Seber capture-recapture estimates. Wildl. Soc. Bull. 31, 814–822.

Moon, L.M., Butler, M., Campbell, L.G., 2022. Evaluation of tagging methods for unique identification of individuals in three aquatic Eurycea Salamander species. Ichthyol. Herpetol. 110, 77–86. https://doi.org/10.1643/h2021042.

Murray, K.A., Skerratt, L.F., Speare, R., McCallum, H., 2009. Impact and dynamics of disease in species threatened by the amphibian chytrid fungus, batrachochytrium dendrobatidis. Conserv. Biol. 23, 1242–1252. https://doi.org/10.1111/j.1523-1739.2009.01211.x.

Musselman, W.C., Worthington, T.A., Mouser, J., Williams, D.M., Brewer, S.K., 2017. Passive integrated transponder tags: review of studies on warmwater fishes with notes on additional species. J. Fish Wildl. Manag. 8, 353–364. https://doi.org/10.3996/122016-JFWM-091.

Newell, D.A., Goldingay, R.L., Brooks, L.O., 2013. Population Recovery following Decline in an Endangered Stream-Breeding Frog (Mixophyes fleayi) from Subtropical Australia. PLoS One 8 (3), e58559.

Nichols, J.D., 1992. Capture-recapture models: using marked animals to study population dynamics. Bioscience 42, 94–102.

Oliveira, B.F., São-pedro, V.A., Santos-barrera, G., Penone, C., Costa, G.C., 2017. Data Descriptor: AmphiBIO, a global database for amphibian ecological traits. Sci. Data 4. https://doi.org/10.1038/sdata.2017.123.

Pickering, C.M., Byrne, J., 2014. The benefits of publishing systematic quantitative literature reviews for PhD candidates and other early career researchers. High. Educ. Res. Dev. 33, 534–548.

Pickett, E.J., Stockwell, M.P., Bower, D.S., Pollard, C.J., Garnham, J.I., Clulow, J., Mahony, M.J., 2014. Six-year demographic study reveals threat of stochastic extinction for remnant populations of a threatened amphibian. Austral Ecol. 39, 244–253. https://doi.org/10.1111/aec.12080.

Poll, C.P., 2009. Wound management in amphibians: etiology and treatment of cutaneous lesions. J. Exot. Pet Med. 18, 20–35. https://doi.org/10.1053/j.jepm.2008.10.005.

Pradel, R., 1996. Utilization of capture-mark-recapture for the study of recruitment and population growth rate. Biometrics 52, 703–709.

Pyke, G.H., 2005. The use of PIT tags in capture-recapture studies of frogs: a field evaluation. Herpetol. Rev. 36, 281–285.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/.

Sannolo, M., Gatti, F., Mangiacotti, M., Scali, S., Sacchi, R., 2016. Photo-identification in amphibian studies: a test of I3S Pattern. Acta Herpetol. 11, 63–68. https://doi.org/10.13128/Acta.
Scheele, B.C., Pasmans, F., Skerratt, L.F., Berger, L., Martel, A., Beukema, W., Acevedo, A.A., Burrowes, P.A., Carvalho, T., Catenazzi, A., et al., 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science (80-.) 363, 1459–1463.
Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, S.L.A., Fischman, D.L., Waller, R.W., 2004. Status and trends of amphibian declines and extinctions worldwide. Science (80-.) 306, 1783–1786.
Therneau, T.M., 2022. A Package for Survival Analysis in R.
Waddle, J.H., Rice, K.G., Mazzotti, F.J., Percival, H.F., 2008. Modeling the effect of toe clipping on treefrog survival: beyond the return rate. J. Herpetol. 42, 467–473.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
Winter, J.D., 1996. Advances in underwater biotelemetry. In: Murphy, B.R., Willis, D.W. (Eds.), Fisheries Techniques, 2nd Edn. American Fisheries Society, Bethesda, Maryland, pp. 555–590.
Wobeser, G.A., 2007. Disease in Wild Animals: Investigation and Management, 2nd ed. Springer-Verlag, Berlin Heidelberg, Germany.
Young, A., McNaught, C.-E., 2011. The physiology of wound healing. Surg. 29, 475–479.