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Short Note

Problems, progress and prospects in marking sympatric newt species with passive integrated transponders

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Abstract. In this essay I evaluate the use of passive integrated transponders (PIT-tags) for individual recognition in five sympatric populations of the large-bodied newts *Triturus cristatus* and *T. marmoratus*. Advice is given on how (not) to insert PIT-tags. With pattern mapping applied in parallel, tag loss was shown to be absent or rare from live animals. Tags on the loose (i.e., without its carrier that presumably died and decomposed) were located in two ponds when these fell dry. Results suggest that aquatic *T. marmoratus* and juvenile *T. cristatus* experience higher natural mortality than adult *T. cristatus*. The long-term research on both species in the département Mayenne, France may be paralleled by research on sympatric *Lissotriton helveticus* and *L. vulgaris*, provided that miniature PIT-tags can justifiably be applied to these small-bodied species.

Keywords: individual marking, *Lissotriton*, pattern mapping, population dynamics, toe clipping, *Triturus cristatus*, *Triturus marmoratus*.

Research into population dynamics is much assisted by the individual recognition of the organisms under study. In amphibians, toe-clipping has extensively been used for this purpose (Murray and Fuller, 2000; Ferner, 2010), but questions have been raised about impact on animal welfare (May, 2004; McCarthy and Parris, 2004). The general conclusion of the discussion that followed is that a balance is to be sought between the risks and disadvantages of marking (with whatever technique) versus the gains in knowledge and understanding (Funk et al., 2005; Phillot et al., 2008; Parris et al., 2010; Grafe et al., 2011; Perry et al., 2011; Soulsbury et al., 2020; Daversa et al., 2024).

Practical limitations of toe-clipping for individual recognition are that the number of combinations is often limiting (Martof, 1953; Woodbury, 1956; Donnelly and Guyer, 1994) and that in salamanders and some frogs shortened toes regrow (Ferner, 2010; Ursprung et al., 2011) rendering the method unsuitable for long-term studies. For species with distinctive patterns photographic identification may be an option but this technique, known as pattern-mapping, is laborious (Arntzen et al., 2004), although progress has been made with automated recognition software in a variety of merchandise (see Matthé et al., 2017 for a review). An efficient yet somewhat expensive alternative is the use

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of Passive Integrated Transponders. These PIT-tags (henceforth 'tags') are glass-encapsulated microchips that, positioned inside the range of a reading bv on device, provide a unique hexadecimal code that offers permanent and unambiguous identification. I here address two practical issues on the use of tags in newts namely 'how are the tags best implanted?' and 'are tags lost by the animals?' This is relevant because tag loss and tagging-induced mortality can introduce biases and decrease precision in parameter estimates, that could though be adjusted for if these effects are accurately quantified (Arnason and Mills, 1981; McDonald et al., 2003; McCarthy et al., 2009). I also report on tags 'on the loose' (i.e., without a carrier) with signals traced when ponds fell dry. Research was carried out on *Triturus cristatus* (the northern crested newt) and *T. marmoratus* (the marbled newt) in the département Mayenne which is located within these species' area of range overlap in the west of France.

Species composition in five study ponds (with pond codes Ang1, 2A1, 2D5, 232 and 278) ranged from *T. cristatus* dominated to *T. marmoratus* dominated in the order Ang1-2A1-232-2D5-278. For brief pond descriptions and research details see Arntzen (2025ab). The capturing of newts was done in spring, with a dip-net till 2009 from when on funnel trapping was the preferred method (Arntzen, 2002, 2025a; Arntzen and Zuiderwijk, 2020). PIT-tagging started in 2003. While the presence of aquatic juveniles in *T. cristatus* appears erratic, *T. marmoratus* and hybrid juveniles appear to be fully terrestrial and were not encountered. The tags used were Trovan ID-100A with a length of 11.5 mm and a diameter of 2.1 mm and occasionally the smaller 'mini' tag ID-100A/1.4 with a length of 8.0 mm and a diameter of 1.4 mm. The weight of an ID-100A tag is 0.09 g, amounting to ca. 0.7% of the body mass in adults and 1.9% in juveniles in the *Triturus* species here studied. Individuals were anaesthetized with Tricaine methane-sulfonate (MS-222, Sandoz) following guidelines (DeNardo, 1995) and tags were inserted with syringes as supplied by the manufacturer (Trovan, https://www.trovan.com/en/products). In a pilot study tags were injected in the lower abdomen (as in fig. 2 in Le Chevalier et al., 2017; fig. 1 in Weber et al., 2019) of six individuals of both species from outside locations. This procedure was swiftly abandoned because the tension in the belly proved too high for the glue (see below) to keep and internal organs (liver or intestines) were sometimes extruded, especially in the bulky *T. marmoratus* females. All individuals were cared for until the wound had healed and released at the place of capture. Subsequently tags were inserted laterally in a position ca. 15 mm posterior to the insertion of the left foreleg (see fig. 1). Upon puncture, care was taken to rotate the (asymmetric) syringe needle point by >90 degrees and to keep it close under the skin in order not to hit the lungs. The wound was closed with Vetbond (3M) surgical glue, but the gel version of the cheaper and more readily available 'super glue' (various manufacturers) was found to be equally effective, although it takes a little longer to set. Newly tagged individuals were kept overnight to inspect their well-being and checked for the presence of the tag. In nine cases the glue had partially loosened so that a new dose had to be applied prior to release and in two cases the tag had to be reinserted. Viscera extrusion was not observed. An X-ray photograph of 13 individuals found dead in the funnel traps (up to five years after they were tagged) showed that the tags had moved internally from the place of insertion to the lower end of the body (fig. 1).

The codes of recaptured individuals were retrieved with a hand-held Trovan LID-560 or LID-573 reader. Data gathering was supplemented with a submersible ANT612-IP68 reader intermittently applied in ponds Ang1 and 232. This device has a detection range of nominally 35 cm and was also used to search on land around the five study ponds and over the bottom of two ponds (ponds 232 and Ang1) when these fell dry in October 2020. On land

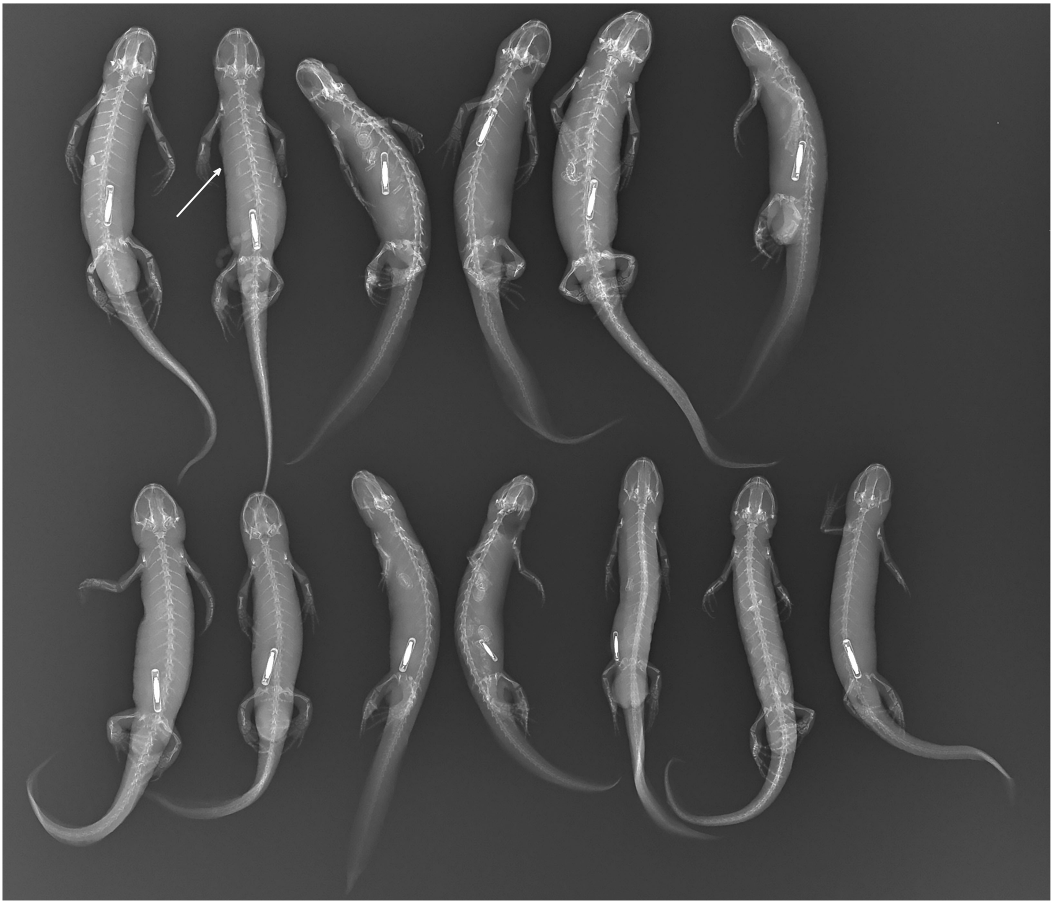

Figure 1. X-ray photographs of 13 *Triturus* newts that died in funnel traps (Arntzen, 2025a). On the top row *T. marmoratus* (numbers 1 to 6) with sexes m, f, f, f, f, m (m = male, f = female). On the bottom row two *T. marmoratus* (7, 8; f, m) and five T. *cristatus* (9-13; f, f, m, m, f). All individuals show an ID-100A tag, except for numbers 10 and 11 that have an ID-100A/1.4 mini transponder and number 12 that is without a tag. The approximate position of PIT-tags insertion is shown by an arrow for individual number 2. Photo by Dirk van de Marel.

the antenna was operated by two people, carried with ropes over their shoulders in a yoke and applying a ca. 70 cm wide lateral swing close to the surface. When operated by a single person the device was slowly pulled over the surface as a sledge. Handling and moving the antenna in the field was straightforward in pasture and a tree plantation but proved problematic in scrub, arable and ploughed fields and in and around hedgerows. More versatile equipment such as the Trovan GR-252 hand held device (or similar equipment, see Blomquist et al., 2008) would possibly do a better job in rugged terrain than the voluminous and heavy antenna (48 × 40 × 4 cm, with the battery amounting to ca. 10 kg).

To investigate potential PIT-tag loss, newly tagged *T. cristatus* in 2014 ($N = 33$) and 2015 ($N = 85$) were photographed for their orange and black belly pattern to allow individual recognition (Hagstrom, 1973; Arntzen and Teunis, 1993) even if tags would have been lost. The numbers of untagged adult newts observed were 19 in 2015, 28 in 2016 and 19 in 2017. None of these 66 individuals could be matched with individuals that were tagged *and* pattern-mapped before, suggesting that they were truly new recruits to the breeding population and not ones that had lost their tag. The absence of tag loss was also reported by Le Chevalier et al. (2017) for *T. marmoratus*. These findings are in contrast with those of Weber et al. (2019) and Jehle and Hödl (1998) who reported an 11-16% PIT-tag loss in *T. cristatus* and *T. dobrogicus*, supporting the view that results may be dependent on species and procedures (see review by Ribas et al., 2022).

Searching the dry bottom of two ponds with the antenna yielded 23 tag signals out of 672, with a higher yield at pond 232 (4.0 %) than in pond Ang1 (2.6%). Because the animals carrying the tag could not be found, it was concluded that it concerned 'loose' tags and – with no evidence for the loss of tags (see above) – these were taken to indicate deceased and decomposed individuals. In pond 232 loose tags were found from seven *T. cristatus* (of which five only seen as juveniles), eight *T. marmoratus* and one interspecies hybrid. Among adults, the relative numbers are not significantly different from the complement of tagged individuals at that time (140 *T. cristatus*, 161 *T. marmoratus* and 29 hybrids) (Fisher's exact test on species, df = 1, $P > 0.05$). However, an about equally heavy aquatic death toll is not in line with expectations from phenological data showing that *T. cristatus* stays in the water for longer than *T. marmoratus*, their annual aquatic periods being ca. five months versus ca. two months (Bouton, 1986; Arntzen, 2003, 2024; Wielstra et al., 2019). This raises the suspicion that *T. marmoratus* is vulnerable in the aquatic phase, just as it succumbs in funnel traps significantly more frequently than *T. cristatus* (0.39% versus 0.12% of captures, Arntzen, 2025a). In the same pond loose tags were traced more frequently from *T. cristatus* juveniles never recaptured (five out of 57, 8.8%) than from the counterpart group (two out of 142, 1.4%) ($P < 0.05$). In pond Ang1 loose tags were also found more frequently from *T. cristatus* juveniles never recaptured (two out of 41, 4.9%) than from the counterpart group (five out of 233, 2.1%), but the difference is not significant ($P > 0.05$). While it cannot be excluded that tagging is especially detrimental to juveniles, e.g. on account of their small size (on average 50 mm snout vent length for one-year olds in spring), it is more likely that the data reflect the lower natural annual survival of juveniles compared to adults (as in Arntzen and Teunis, 1993; see also Jehle and Hödl, 1998).

Despite a ca. eight-hour effort, no PIT-tag signals were retrieved from around the ponds. This is surprising because radio-tracking data indicate that *Triturus* newts do not move far from the ponds and that is where I searched (Jehle, 2000; Jehle and Arntzen, 2000). Results also compare negatively to e.g. those by Ryan et al. (2014) who detected 74 terrestrial tags in 119 hours of survey time in the salamander *Ambystoma laterale*. The searching for terrestrial newts with trained dogs may be a valuable alternative (Grimm-Seyfarth, 2022; Glover et al., 2023; McKeague et al., 2024).

Closing considerations

In my experience inserting PIT-tags in the posterior abdomen of *T. marmoratus* causes serious harm, when untreated possibly leading to death and I advise against this procedure. Insertion at the anterior side of the body worked well, but the procedure requires a little practice and would be tricky if animals were not immobilized, i.e., anesthetised (see Le Chevalier et al., 2017 versus Ribas et al., 2022).

Triturus cristatus is a newt species especially amenable to demographic analyses because juveniles can be found in the water, although few studies have taken this asset to their advantage (Arntzen and Teunis, 1993; Kupfer and Kneitz, 2000). Indeed, the present study could have been improved by dedicated searches for aquatic juveniles after the breeding season, especially in years that juvenile cohorts were substantial. The species (and others with easy to recognize individual colouration patterns) is also amenable to study effects of invasive marking. Following this research line, Jehle and Hödl (1998) observed no significant differences in survival and body condition for PIT-tagged versus photographed-only individuals of *T. dobrogicus*, to which it must, however, be noted that their groups were not entirely comparable.

Further research promise is shown in addressing the comparative demography of the small-bodied newts *Lissotriton helveticus* and *L. vulgaris*. In western France, these species are frequently found in sympatry with *T. marmoratus* and *T. cristatus* while displaying parallel ecological preferences (Evrard et al., 2022). A pilot study is needed to investigate the applicability of mini and 'nano' tags on these delicate creatures.

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