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Portable PIT detector as a new tool for non-disruptively locating individually tagged amphibians in the field: a case study with Pyrenean brook salamanders (*Calotriton asper*)

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Abstract. Passive Integrated Transponder (PIT) telemetry has recently been adapted for locating PIT-tagged fish in shallow waters using portable detectors. In the present study, we tested this method for adult amphibians (Pyrenean brook salamanders, *Calotriton asper*) PIT-tagged with 12-mm transponders in a headwater rocky stream. PIT telemetry performances were compared with a conventional hand-capture method, i.e. visual searching and overturning movable stones in the streambed. The mean efficiency of the portable detector (88.2% ± 5.2, s.e.) was significantly higher than hand-capture (51.1% ± 6.4) and the operator progressed, on average, four times faster. Time efficiencies were, on average, 0.92 (±0.15) and 0.12 (±0.04) adult salamanders per minute with the portable detector and by hand-capture, respectively. The efficiency of the portable detector was negatively correlated with the percentage of large stones on the streambed. The time needed to prospect a section was positively correlated with the abundance of PIT-tagged adult salamanders, spring inlets and undercut banks. Because PIT telemetry is less disruptive than hand-capture for both salamanders and their habitat, it is a promising and non-disruptive method for developing studies on the ecology and management of amphibians in shallow waters.

Introduction

Since their first use in the mid-1980s, passive integrated transponder (PIT) tags have enabled innovative investigations into numerous aspects of animal biology, including behaviour, physiology, game management, conservation and life history (Gibbons and Andrews 2004). PIT tags provide millions of unique alphanumeric codes in relatively small and low-weight biocompatible glass capsules that can be detected using hand readers. They have a long lifespan (>10 years) and a relatively low cost (Hammer and Blankenship 2001; Gibbons and Andrews 2004). Using PIT tags for mark–recapture experiments with wild animals began with fisheries studies (reviewed in Lucas and Baras 2000), specifically with salmonids (Prentice *et al*. 1990*a*). The use of PIT tags for mark–recapture of animals in field situations subsequently expanded to a wide variety of taxa such as crustaceans, amphibians, reptiles, birds and mammals (reviewed by Gibbons and Andrews 2004).

In the 1990s, fisheries biologists designed stationary PIT antennae to automatically record fish passage through fishway orifices (Prentice *et al*. 1990*b*; Castro-Santos *et al*. 1996), through stream-wide antennae (Morhardt *et al*. 2000; Barbin Zydlewski *et al*. 2001), or above flat-bed antennae laid on the stream bed (Armstrong *et al*. 1996; Alanara and Brannas 1997). The use by ichthyologists of these stationary antennae that continuously detect PIT-tagged individuals has proven effective for the study of fish management, behaviour, habitat selection and life history (e.g. Brännäs *et al*. 1994; Metcalfe *et al*. 1999; Cucherousset *et al*. 2006). More recently, PIT technology has been adapted for locating PIT-tagged fish in shallow waters. Termed 'PIT telemetry', the system consists of using portable detectors to locate PIT-tagged fish and actively monitor their movements without the need for handling. Several devices have been developed and field-tested to work with 23-mm (Morhardt *et al*. 2000; Roussel *et al*. 2000; Barbin Zydlewski *et al*. 2001; Hill *et al*. 2006) and 12-mm PIT tags (Cucherousset *et al*. 2005*a*; Keeler *et al*. 2007). These technological developments have greatly contributed to the understanding of complex aspects of fish and crayfish ecology, behaviour and management (Cunjak *et al*. 2005; Quintella *et al*. 2005; Bubb *et al*. 2006; Cucherousset *et al*. 2007*a*).

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Many amphibian species are declining worldwide (Stuart *et al.* 2004). Their populations are often monitored through pitfall traps associated with drift fences, funnel traps, or net- and hand-capture (Heyer *et al.* 1994; Brown 1997; Lecis and Norris 2003). Hand-capture is commonly used in mark–recapture studies to locate headwater stream amphibians and involves searching through unconsolidated stream substrate ('rubble-rousing': Barr and Babbitt 2001; Steele *et al.* 2003) or visually searching an area while overturning movable surface objects ('light touch': Quinn *et al.* 2007). These conventional methods are time-consuming and could be quite intrusive because they require disturbance of the stream and handling of the amphibians.

In the present study, we aimed to extend the use of PIT telemetry by determining the efficiency of locating PIT-tagged adult amphibians in the wild. Indeed, several studies involving amphibians have concluded that PIT-tagging has virtually no effects on their survival, growth and body condition (e.g. Brown 1997; Jehle and Hodl 1998; Ott and Scott 1999; Perret and Joly 2002), but there has been no published attempt at using PIT telemetry with amphibians. Our specific objectives were to (1) measure the performance (detection and time efficiencies) of PIT telemetry to locate PIT-tagged adult Pyrenean brook salamanders (*Calotriton asper*) in a small and shallow rocky stream, (2) compare PIT telemetry to a conventional hand-capture method, and (3) evaluate the effect of habitat characteristics on the performance of PIT telemetry in locating stream amphibians.

Materials and methods

Study area and species

The study was undertaken in a small tributary of the River Oriège, a torrential stream in the Pyrénées Mountains (south-west France) that flows into the River Ariège at 815 m above sea level. The study was conducted in the upstream section of the River Oriège (1480 m above sea level), an area that is surrounded by a 35-ha homogenous grassland composed of *Poaceae* and *Equisetum* sp., and which belongs to a 4000-ha National Reserve (Réserve Naturelle d'Orlu, detailed in Cucherousset *et al.* 2007*b*). The tributary was selected because adult Pyrenean brook salamanders have been studied there for several years (Baraillé *et al.* 2006). The tributary was 153 m long and shallow (mean depth = 6.5 cm ± 0.7 s.e., maximum depth = 19 cm) with a mean width of 2.4 m (±0.3). For the present study it was divided into 10 sections of ~30 m^2 each.

The Pyrenean brook salamander occurs in an altitudinal range from 140 to 2500 m above sea level in the Pyrénées Mountains. Although this endemic and rare species is listed on Annex II of the Bern Convention and Annex IV of the European Habitats Directive, it is locally common in the east and the central part of the Pyrénées Mountains. This species exhibits a life cycle with a relatively long aquatic period in lotic habitats, especially in cold rocky streams. Prebreeding migrations of adults towards streams generally starts in mid-May and they leave the streams in September after reproduction. Highest abundances of adults are recorded from June to August (Baraillé *et al.* 2006).

Tagging procedure

In total, 265 adults (138 males and 127 females) were hand-captured (see details below) and PIT-tagged from May 2004 to December 2005 by Baraillé *et al.* (2006). Each individual was anaesthetised by immersion in a phenoxy-ethanol solution (0.5%), measured for snout–vent length (SVL) to the nearest millimetre, weighed to the nearest 0.1 g and PIT-tagged. Tagging consisted of a PIT tag injection in the ventral part of the peritoneal cavity with a modified hypodermic syringe and a 2.7-mm-diameter sterile needle (e.g. Cucherousset *et al.* 2005*b*). The transponders used were 11.5 mm long and 2.1 mm in diameter (Trovan ID100, EID Aalten B.V., Aalten, The Netherlands); they weighed 0.100 g in air and 0.058 g in water (Baras *et al.* 2000). PIT-tagged adults were then released in the stream following recovery. Mean SVL was 71.5 mm (±0.3 s.e.) for males and 71.3 mm (±0.4 s.e.) for females. Males and females weighed 8.8 g (±0.1 s.e.) and 7.6 g (±0.1 s.e.), respectively. These individuals were bigger than those used in previous studies in which amphibians were PIT-tagged (e.g. Ott and Scott 1999; Holenweg and Reyer 2000; Perret and Joly 2002), ensuring that PIT-tagging was unlikely to affect them. In June 2007 (18 months after tagging), we tracked PIT-tagged adult salamanders in natural conditions of spatial distribution, density and localisation in each of the 10 sections of the tributary to test the performance of the portable detector. Although we did not aim to measure mortality and/or PIT tag rejection in this study, we did expect a portion of the PIT tags to be shed into the stream as a result of mortality or tag rejection, providing us with the additional opportunity to determine our ability to locate free PIT tags on the stream bed.

Tracking procedures

We performed the PIT-tracking surveys using a portable PIT detector composed of a RFID reader (LID 650; EID Aalten B.V., Aalten, The Netherlands) interfaced with a LCD screen and powered by a 12-V battery. The reader was connected to a waterproof antenna (ANT 612; EID Aalten B.V., Aalten, The Netherlands) that was mounted on a 3-m-long aluminium pole. The maximum tag-detection distances range from 30 to 36 cm, depending on the horizontal or vertical orientation of the PIT tag. A thorough description of the portable detector is given in Cucherousset *et al.* (2005*a*). We surveyed sections of the tributary in 2007, at a time of year when the abundance of adult salamanders in the stream was considered to be the highest (Baraillé *et al.* 2006): four sections on 22 June, two on 23 June, and four on 29 June. We enclosed each section with barrier nets (5-mm mesh) to prevent salamander displacement among sections during monitoring.

We conducted three successive monitoring runs per section. The first run was devoted to PIT tracking. The operator moved upstream in the section by walking on the stream bank or wading and moving the antenna just above the water surface from one bankside to the other ('sweeping technique': Cucherousset *et al.* 2005*a*). We also scanned a 50-cm width of bank along the entire section in search of PIT tags in the undercut banks. The operator was not aware of the number of PIT-tagged salamanders nor of free PIT tags that might be encountered per section. When the antenna detected a PIT tag, a piezoelectric buzzer sounded a loud tone to alert the operator, and the tag code was displayed on the

LCD screen. A second operator recorded the PIT code on field sheets. The tag code and hour of detection were also automatically recorded in the reader's data logger. Data from the data logger were downloaded onto a computer after the tracking session, thus enabling double-checking of the list of detected PIT tags.

The sweeping technique might lead to a lower detection of cluster tags since it is impossible to simultaneously detect multiple tags in the antenna field (e.g. Castro-Santos *et al.* 1996). To address this issue, the operator manoeuvred the antenna around the area of each detected tag using different angles, so that the 'strongest' tag signal could be detected for each tag in a cluster (Linnansaari *et al.* 2007).

The second run was conducted just after the end of the first run and consisted of a hand-capture survey, as commonly practiced in studies of adult amphibians in running sections of shallow streams (e.g. Grant *et al.* 2005; Quinn *et al.* 2007). This was done by two operators who did not participate in the first run, and were not aware of portable detection records. Operators waded or walked along the section in an upstream direction, one on each side of the stream, flipping over rocks carefully to hand-capture the salamanders in the wetted channel, and then returning the rocks to their original position. Operators also thoroughly checked for salamanders in all undercut banks and spring inlets (i.e. small and rocky areas in the banks with interstitial water flowing in the tributary). Our hand-capture survey was designed for studying adult salamanders only and so was similar to the 'light-touch' survey developed by Quinn *et al.* (2007), i.e. a 'rubble-rousing' procedure but without searching in the deep substrate or sifting the fine substrate. We checked captured adult salamanders for PIT tag presence and, if detected, recorded the PIT code. After capture, salamanders were held outside the tracked section in oxygenated water until the end of the monitoring experiment.

The third and final run was conducted just after the end of the second run and consisted of using the two techniques in conjunction (PIT tracking and hand-capture) to collect all the remaining PIT-tagged salamanders and free PIT tags in each section. When we detected a PIT tag, we thoroughly checked the area in search of salamanders. When we did not find a PIT-tagged salamander, we dug the substratum in search of free PIT tags and removed each tag individually. This third run lasted until we removed all PIT-tagged salamanders and free PIT tags from the section, i.e. until we detected no more PIT tags when sweeping the experimental area and until we found no more salamanders by overturning all movable stones. At the end of the tracking, we released all salamanders at the precise location of their capture. Afterwards, we determined the proportion of hand-captured PIT-tagged adult salamanders and free PIT tags that had been detected or missed with the portable detector during the first run.

Habitat characteristics

We measured habitat characteristics that have already been identified as factors influencing the performance of portable PIT detectors (e.g. Keeler *et al.* 2007). Three transects were performed in each section to measure river width to the nearest 10 cm and the bank undercut distance to the nearest 5 cm. Along each transect, points were performed every 50 cm to measure depth to the nearest centimetre and to visually determine the percentage cover by large stones (200–600 mm in diameter, expressed as a percentage) on the streambed. We categorised water velocity into null, low (>0–0.7 cm s^{-1}), medium (0.8–4.6 cm s^{-1}), high (4.7–20.0 cm s^{-1}) and very high (>20.0 cm s^{-1}). We also noted the number of spring inlets along the banks.

Data analyses

After determining the status of each PIT tag – free or in a salamander – in each section, we calculated several variables relating to PIT telemetry and hand-capture performance. We calculated the total number of PIT-tagged adult salamanders (N_T) as:

$$N_T = N_2 + N_3$$

where N_2 is the number of PIT-tagged adult salamanders caught by hand during the second run and N_3 is the number of PIT-tagged adult salamanders caught during the third run.

We calculated the efficiency of the portable detector to locate PIT-tagged adult salamanders (E_{PIT}, expressed in %) as:

$$E_{PIT} = N_1/N_T \times 100$$

where N_1 is the number of PIT-tagged adult salamanders located with the portable detector during the first run.

We calculated the efficiency of hand-capture of adult salamanders (E_{HAND}, expressed as a percentage) as:

$$E_{HAND} = N_2/N_T \times 100$$

We calculated the efficiency of the portable detector to locate free PIT tags (E_{PIT*}, expressed as a percentage) as:

$$E_{PIT*} = N_1* / N_3*$$

where N_{1*} is the number of free PIT tags located with the portable detector during the first run and N_{3*} the number of free PIT tags removed from the substratum during the third run.

Finally, we measured the time consumption on the first (TC_{PIT}) and second (TC_{HAND}) runs (in minutes). We then calculated the time efficiency (expressed in PIT-tagged adult salamanders per minutes) of the portable detector (TE_{PIT}) and of hand-capture (TE_{HAND}) to locate a PIT-tagged salamander using the formulae:

$$TE_{PIT} = N_1/TC_{PIT} \quad \text{and} \quad TE_{HAND} = N_2/TC_{HAND}$$

We tested differences between E_{PIT}, and E_{HAND}, between E_{PIT} and E_{PIT*}, and between TE_{PIT} and TE_{HAND} using Wilcoxon signed-rank tests. We examined the relationships between habitat characteristics, the number of PIT-tagged salamanders and the performances of the portable detector using Pearson correlation analyses. We adjusted the significance level of multiple comparisons using the false-discovery rate method to minimise Type I errors (Storey 2002). The false-discovery rate method provides Q-values that are interpreted as P-values. We set a significance level at $\alpha = 0.05$.

Results

Overall, N_T was highly variable between sections, ranging from 2 to 48 PIT-tagged adult salamanders and averaging 13.1 (±4.7 s.e., see details in Appendix 1). Mean E_{PIT} was 88.2% (±5.2), ranging from 50 to 100% (Fig. 1). We measured the lowest E_{PIT} in one of the two sections that contained only two PIT-tagged salamanders (i.e. one PIT-tagged salamander was not detected during the first run). The next lowest E_{PIT} was 73.3%. In total, 131 PIT-tagged salamanders were present in the whole tributary, 86.3% ($n = 113$) being detected by the portable detector during the first run. Among these 113 PIT-tagged adult salamanders, four were recorded on the data logger without being noted by the operators during the tracking. On average, we needed 10.4 min (±6.4) to track the 30 m^2 of each section during the first run (TC_{PIT}). Mean TE_{PIT} was 0.92 PIT-tagged salamander per minute (±0.15).

Mean E_{HAND} was 51.1% (±6.4), ranging from 14.3 to 73.3%, and was significantly lower than E_{PIT} ($Z = 2.668$, $P = 0.008$, $n = 10$) (Fig. 1). On average, we needed 39.9 min (±11.7) to perform the second run by hand (TC_{HAND}) in each section and mean TE_{HAND} was 0.12 PIT-tagged adult salamanders per minute (±0.04). TE_{HAND} was significantly lower than TE_{PIT} ($Z = 2.803$, $P = 0.005$, $n = 10$) (Fig. 2). Seven of the 10 sections contained a total of 43 free PIT tags, N_{3*} ranging from 1 to 10. The mean E_{PIT*} was 89.7% (±5.51), ranging from 62.5 to 100% and did not significantly differ from E_{PIT} ($Z = 0.762$, $P = 0.446$, $n = 7$) (Fig. 1).

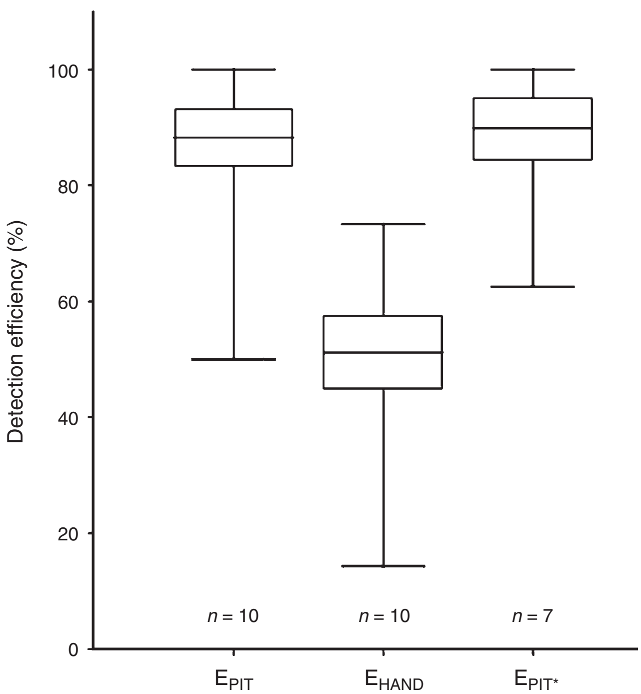

Fig. 1. Efficiency (%) of the portable detector to locate PIT-tagged Pyrenean brook salamanders (*Calotriton asper*) (E_{PIT}) and free PIT tags (E_{PIT*}), and the efficiency of conventional hand-capture method (E_{HAND}) in 10 sections of a tributary of the River Oriège (south-west France) in June 2007. The central lines represent the means, the boxes represent the means with the standard errors and the whiskers represent the minimum and maximum values.

E_{PIT} was negatively and significantly correlated with the percentage of large stones ($r = -0.726$, $Q = 0.031$, $n = 10$) (Table 1). TC_{PIT} was strongly and positively correlated with N_T ($r = 0.973$, $Q < 0.001$, $n = 10$), to the number of spring inlets ($r = 0.935$, $Q < 0.001$, $n = 10$) and to the undercut distance in banks ($r = 0.764$, $Q = 0.024$, $n = 10$) (Table 1).

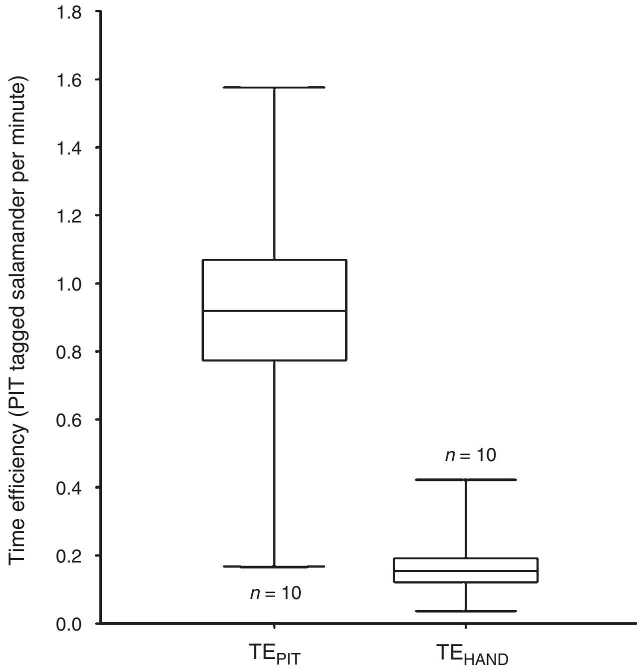

Fig. 2. Time efficiency (in PIT-tagged salamanders per minute) of the portable detector (TE_{PIT}) and conventional hand-capture method (TE_{HAND}) to locate PIT-tagged Pyrenean brook salamander (*Calotriton asper*) in 10 sections of a tributary of the River Oriège (south-west France) in June 2007. The central lines represent the means, the boxes represent the means with the standard errors and the whiskers represent the minimum and maximum values.

Table 1. Correlations between the performance of the portable detector and environmental characteristics

Coefficients (*r*) and probabilities (*Q*-values) for the Pearson correlations between the performances of the portable detectors, the total number of PIT-tagged salamanders (N_T) and the environmental characteristics in the 10 sections of a tributary of the River Oriège (south-west France) in June 2007. Performances estimated are the efficiency of the portable detector to locate PIT-tagged Pyrenean brook salamander (*Calotriton asper*) (E_{PIT}, in %) and time consumption of the first run with the portable detector (TC_{PIT}, in minutes). Significant values are shown in bold

Variables	E_{PIT}		TC_{PIT}	
	r	*Q*	*r*	*Q*
N_T	–0.117	0.409	**0.973**	**<0.001**
Width (m)	–0.556	0.114	–0.126	0.409
Depth (cm)	–0.267	0.360	–0.451	0.195
Velocity (categorical)	–0.420	0.202	–0.132	0.409
Large stone (%)	**–0.726**	**0.031**	0.583	0.109
Undercut distance under banks (cm)	0.027	0.480	**0.764**	**0.024**
No. of spring inlets	–0.212	0.396	**0.935**	**<0.001**

Discussion

PIT telemetry appears to be an effective and efficient method for locating PIT-tagged amphibians in shallow streams and can thus be useful for monitoring organisms of high conservation status in nature with limited impacts on the animals and the habitat. Indeed, we found in field conditions that PIT telemetry was more efficient and less time-consuming than a standard hand-capture method to locate adult salamanders tagged with small (12-mm) PIT tags. The detection efficiency of PIT-tagged salamanders was similar to the efficiency of detection of free PIT tags, suggesting that the operator did not specifically induce fleeing behaviour of adult salamanders. The performance of the portable detector (efficiency or time consumption) was affected by habitat characteristics, notably the presence of spring inlets and large stones on the stream bed that hamper the operator while manoeuvring the portable PIT detector.

Since the development of portable detectors for 12-mm PIT tags, several studies have tested their performance with free PIT tags and PIT-tagged fish. Here, the efficiency of the portable detector to locate free PIT tags (89.7% ± 5.5) was similar to the results reported by Bubb *et al*. (2002), who detected >90% and >80% of free PIT tags placed beneath rocks and in burrows, respectively. While searching for PIT-tagged salamanders with the portable detector, the detection efficiency (88.2% ± 5.2) falls within the upper values reported for different fish species using 12-mm PIT tags. The lowest detection efficiencies have been reported for one-year-old brown trout (*Salmo trutta*) and northern pike (*Esox lucius*) with 69% and 71.4%, respectively (Cucherousset *et al*. 2005*a*, 2007*a*). When using a portable detector on young-of-the-year brown trout and slimy sculpin (*Cottus cognatus*), >80% efficiencies have been reported (Cucherousset *et al*. 2005*a*; Keeler *et al*. 2007). Finally, the highest detection efficiencies have been reported for juvenile sea lamprey (*Petromyzon marinus*), with 87% of metamorphosed juveniles and 94.5% of ammocoete larvae being located with a portable detector (calculated from Quintella *et al*. 2005). In the present study, we measured a high detection efficiency close to the values obtained for sea lamprey (Quintella *et al*. 2005) and for free PIT tags (Bubb *et al*. 2002), probably because these species are generally hidden under rocks or buried in the substratum, and do not flee during tracking. Conversely, pelagic fish such as trout or pike can quickly swim away when the operator performs the survey, leading to lower detection efficiency for mobile animals. A more quantitative approach to determine how species' fleeing behaviour and/or swimming ability affect the efficiency of PIT telemetry is needed, both within (e.g. anurans and urodeles) and among (e.g. crayfish, fish and amphibians) animal groups.

The specific behaviour of a salamander species can also alter the performances of the portable detector. Adult Pyrenean brook salamanders are known to preferentially use microhabitats with a large substrate size (Clergue-Gazeau and Martinez-Rica 1978). Here, the efficiency of the portable detector to locate PIT-tagged adult salamanders was negatively affected by the percentage of large stones in the section, as it was also observed by Keeler *et al*. (2007) with benthic fish species. This result is probably the consequence of stones, root masses or woody debris in the tracking area hampering the operator while trying to get the antenna close enough to PIT-tagged animals to detect them (Roussel *et al*. 2000; Bubb *et al*. 2002). In the present study, the efficiency of the portable detector was not affected by the undercut distance under the banks, and this variable was positively correlated with the time needed to survey a section with the portable detector. Similarly, the density of PIT-tagged adult salamanders increased the time needed to survey a section. We observed some adult salamanders displaying an aggregative distribution, with up to five individuals being localated within the same 0.04-m^2 area in a small spring inlet. Nevertheless, the efficiency of the portable detector to locate each individual tag in a cluster of PIT-tagged adult salamanders was high, even if the antenna-manoeuvring technique used to detect closely located tags was more time-consuming (see Methods section). Importantly, four PIT-tagged adult salamanders detected during the first run were recorded on the data logger but were not noted by the operators during the tracking. This is likely to occur in clusters of PIT-tagged adult salamanders when one of the codes is displayed for a very short period on the LCD screen before another tag is detected, indicating that the data logger is an important backup to recording codes in the field that also minimises transcription errors.

The portable PIT-telemetry method tested in this study is limited to shallow-water streams and may be less efficient when water depth is >30 cm (i.e. maximum reading distance of the detector), for instance during high flow levels or in deeper pools. Adult Pyrenean brook salamanders are known to bury themselves, hide in deep holes under the bank or to use rodent cavities (Despax 1923; Clergue-Gazeau and Martinez-Rica 1978). Consequently, they could place themselves out of the detection range of the antenna. Furthermore, complementary studies should be conducted to determine the performance of PIT telemetry in non-aquatic habitats during the terrestrial phase of amphibians but also with terrestrial animals.

PIT telemetry using 12-mm tags appears to be an appropriate tool for locating and studying amphibians during their aquatic phase. The technique is a useful alternative to standard radio-telemetry in small-scale environments because 12-mm PIT tags can be implanted in smaller-bodied animal species, with virtually no negative impacts on animals provided the organism is sufficiently large (Acolas *et al*. 2007). By contrast to radio-telemetry, where sample sizes are often limited by financial considerations, a large number of individuals over a wide size range can be PIT-tagged (Bubb *et al*. 2006). PIT technology has been shown to be effective at facilitating mark–recapture studies, with a reduced number of tagged individuals required and higher detection efficiency than conventional tools (Skalski *et al*. 1998; Roussel *et al*. 2000; Keeler *et al*. 2007). Additionally, PIT telemetry is less time-consuming for field operators and less disruptive for the animal by reducing potential injuries due to repeated handlings (Keeler *et al*. 2007).

Locating animals using PIT telemetry suffers from two disadvantages compared with conventional hand capture. First, early life stages cannot be studied because tag/bodyweight ratio should not exceed a specific threshold, to avoid impacting on the behaviour, growth or survival of marked organisms (Acolas *et al*. 2007). To avoid this problem, only adults have been PIT-tagged here, as reported elsewhere (>40 mm SVL: Holenweg and Reyer 2000; Perret and Joly 2002). However, we are not aware of any formal attempt to determine the minimum size at PIT-tagging of salamanders. Such investigations are needed to determine whether PIT telemetry can be used efficiently with earlier life stages and other species with smaller adult body-size. Because PIT-tags are among the smallest tags available, PIT telemetry still enables the study of earlier life stages than conventional telemetry, as has already been demonstrated for fish (Baras *et al*. 2000; Acolas *et al*. 2007; Cucherousset *et al*. 2007*a*).

Second, a high number (43 of 265) of free PIT tags was detected. PIT-tagging has virtually no impact on the survival of adult amphibians (Perret and Joly 2002) provided they are sufficiently large (see above). Furthermore, Pyrenean brook salamanders have a long lifespan, and it is thus unlikely that free PIT tags were due to mortality. Rather, we suspect a high tag rejection rate (16.2%) in our study compared with the range of values already reported for amphibians (0.0, 1.7, 10.5 and 16.9% reported in Brown 1997; Jehle and Hodl 1998; Ott and Scott 1999; Perret and Joly 2002 respectively). The implementation method (hypodermic injection (Perret and Joly 2002) or surgical technique with incision (Ott and Scott 1999)) and tag location (dorsal (Brown 1997) or ventral (Perret and Joly 2002)) might influence rejection rate and the most efficient implementation method should be determined for each species. Furthermore, differences in species' ecology and behaviour can account for differential PIT tag loss (Gibbons and Andrews 2004) and Pyrenean stream salamanders might exhibit particular characteristics that increase tag loss. Consequently, experimental studies should be conducted to determine the most efficient implementation method and tag location for each species to ensure a high retention rate during field surveys.

PIT telemetry has minimal impact on salamander habitats because tagged animals can be located without moving stones on the streambed. Such considerations are important for efficient and management-oriented monitoring of threatened species (Nichols and Williams 2006). Therefore, PIT telemetry provides a useful tool that could be applied to study several aspects of animal ecology (e.g. microhabitat use, displacement, site fidelity, mate choice) for a large range of small-bodied animal species (e.g. amphibians, reptiles, small mammals, molluscs, crustaceans, large insects) that display fine-scale movements in restricted habitats. This technology can be used to study life-history traits during the entire lifespan of an organism and to investigate mortality causes since PIT tags have no battery and can be detected after the death of the animal (Gibbons and Andrews 2004; Cucherousset *et al*. 2007*a*). A combination of PIT telemetry and conventional hand capture is needed in mark–recapture studies, for instance when animals have to be measured, when survival and reproduction parameters have to be estimated, or to regularly mark new individuals.

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Appendix 1. Variables relating to Passive Integrated Transponder (PIT) telemetry and hand-capture performances

N_T, the total number of PIT-tagged Pyrenean brook adult salamanders (*Calotriton asper*); N_1, the number of PIT-tagged salamanders located with the portable detector during the first run; TC_{PIT}, the time consumption of the first run (in minutes); E_{PIT}, the efficiency of the portable detector to locate PIT-tagged salamanders (as a percentage); TE_{PIT}, the time efficiency of the portable detector (in PIT-tagged salamanders per minute); N_2, the number of PIT-tagged salamanders caught by hand during the second run; TC_{HAND}, the time consumption of the second run (in minutes); E_{HAND}, the efficiency of hand-capture of salamanders (as a percentage); TE_{HAND}, the time efficiency of hand-capture (in PIT-tagged salamanders per minute); N_{1*}, the number of free PIT tags located with the portable detector during the first run; N_{3*}, the number of free PIT tags removed from the substratum during the third run; E_{PIT*}, the efficiency of the portable detector to locate free PIT tags. See details in the Methods section for calculations. n.a., not available

Section	N_T	N_1	TC_{PIT}	E_{PIT}	TE_{PIT}	N_2	TC_{HAND}	E_{HAND}	TE_{HAND}	N_{1*}	N_{3*}	E_{PIT*}
A	7	7	7	100.0	1.00	5	37	71.4	0.14	6	7	85.7
B	30	22	16	73.3	1.38	7	57	23.3	0.12	8	10	80.0
C	15	15	11	100.0	1.36	11	26	73.3	0.42	5	8	62.5
D	8	7	9	87.5	0.78	5	19	62.5	0.26	9	9	100.0
E	2	2	6	100.0	0.33	1	10	50.0	0.10	0	0	n.a.
F	48	41	26	85.4	1.58	33	137	68.8	0.24	8	7	100.0
G	2	1	6	50.0	0.17	1	28	50.0	0.04	1	1	100.0
H	7	6	9	85.7	0.67	4	45	57.1	0.09	1	1	100.0
I	7	7	10	100.0	0.70	1	24	14.3	0.04	0	0	n.a.
J	5	5	4	100.0	1.25	2	16	40.0	0.13	0	0	n.a.