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USE OF FLUORESCENT PIGMENTS AND IMPLANTABLE TRANSMITTERS TO TRACK A FOSSORIAL TOAD (*PELOBATES FUSCUS*)

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Compared to other vertebrate groups, movement patterns and microhabitat use in amphibians has been little studied. The two goals of this study were (1) to compare two different methods of tracking amphibians (implantable transmitters and fluorescent pigments); and (2) to characterize movement patterns and habitat use in the nocturnal, fossorial spadefoot toad (*Pelobates fuscus*). A fluorescent pigment method was useful for microhabitat studies, as trails could be detected in all kinds of terrestrial habitats, even under wet conditions. Using this method it was possible to trace complete nocturnal movement patterns (maximum distance moved: 73 m). Implantable transmitters were particularly appropriate for fossorial species, such as *Pelobates fuscus*. Diel home range and microhabitat preferences were more precisely defined using a combination of telemetry and pigments. In addition, the vertical component of habitat use could be assessed. The spadefoot toad was more likely to use areas of bare soil or short vegetation and seemed to avoid shrub-covered areas. Mean distance moved between two successive burrows was higher in females (22.9 m) than in males (12.9 m).

Key words: amphibian movement, fluorescent pigments, microhabitats, *Pelobates fuscus*, radiotelemetry

INTRODUCTION

Patterns of animal movement can provide useful information on migration, dispersal, homing activity, activity area, and site selection for reproduction. Given world-wide declines in amphibian populations (Barinaga, 1990; Halliday, 1998; Pechmann *et al.*, 1991; Wake, 1991), herpetologists have taken a special interest in amphibian movements and habitat use (Demaynadier & Hunter, 1998; Dodd & Cade, 1998; Gibbs, 1998; Sjogren-Gulve, 1998). Management practices may enhance amphibian dispersal (Seabrook & Dettmann, 1996), enabling new populations to establish. Alternatively, management can be used to restrict dispersal and thereby minimize mortality by road-kill.

Small body size technically limits the study of movement by small vertebrates. With amphibians, capture-mark-recapture studies (e.g. Clarke, 1974; Haapanen, 1974; Denton & Beebee, 1992) are labour-intensive outside the breeding season and often provide limited data on individual, day-to-day movements. Radio-isotope tracing has been used occasionally (e.g. Ashton, 1994; Barbour *et al.*, 1969), and a tracking device (a sewing machine bobbin in a holder tied around the waist) was used with large, non-burrowing individuals for short study periods (e.g. Dole, 1965; Sinsch, 1987; Sinsch, 1990). Although the development of small and lightweight radio-tracking systems seems promising (Nuland & Claus, 1981), attaching the transmitter is problematic (van Gelder, Aarts & Staal, 1986*a*; Golay, 1996; Tramontano, 1997). External transmitters are especially difficult to use with burrowing species, so implantable ones may be more practical (Madison, 1997; Olders, van Gelder & Krammer, 1985).

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Telemetry studies of amphibians are growing in number. External transmitters with various modes of attachment have been used on anurans such as *Bufo americanus* (Tester, 1963 quoted in van Nuland & Claus, 1981), *B. marinus* (Seabrook & Dettmann, 1996), *B. bufo* (van Gelder *et al.*, 1986*a*; van Nuland & Claus, 1981), *B. viridis* (Baumgart, unpublished data), *B. calamita* (Golay, 1996), *Rana temporaria* (Fiorito *et al.*, 1994; Tramontano, 1997), *Rana muscosa* (Matthews & Pope, 1999) and *Buergeria buergeri* (Fukuyama, Kusano & Nakane, 1988). Oldham & Swan (1992) forced adult *Rana temporaria* and *Bufo bufo* to swallow transmitters, as did Pearson & Bradford (1976) with *Bufo spinulosus*. Implantable transmitters were used in anurans, such as *Bufo bufo* (van Gelder *et al.*, 1986*b*; Olders *et al.*, 1985), *B. americanus* (Werner, 1991), *B. spinulosus* (Sinsch, 1991), *B. calamita* (Sinsch, 1992), *B. canorus* (Martin, unpublished data), *Rana clamitans* (Lamoureux & Madison, 1999) and *Hoplobatrachus occipitalis* (Spieler & Linsenmair, 1998), and in urodeles such as *Cryptobranchus alleganiensis* (Blais, 1996 quoted in Madison, 1997; Stouffer *et al.*, 1983), *Ambystoma maculatum* (Madison, 1997), *A. tigrinum* (Madison, 1998; Madison & Farrand, 1998), *A. gracile* (Stringer, 1997), *Salamandra lanzai* (Riberon, Miaud & Guyétant, 1997) and *Triturus cristatus, T. marmoratus* and their hybrids (Jehle & Arntzen, 2000). Even larval *Dicamptodon tenebrosus* have been radio-tracked (Colberg *et al.*, 1997).

Even so, telemetry studies have their limitations. For example, the precision of tracking depends on the number of location points, and the presence of an observer may influence the behaviour of the studied animal. One approach that may circumvent many difficulties is the use of fluorescent pigments that rub off onto the ground when the animal moves. These have been used for indirect visual tracking of small, nocturnal

rodents (Duplantier *et al.*,, 1984; Frantz, 1972; Lemen & Freeman, 1985), lizards (Fellers & Drost, 1989; Dodd, 1992), hatchling turtles (Butler & Graham, 1993) and tortoises (Blankenship, Bryan & Jacobsen, 1990). Recently, this method has been adapted for, and tested on, amphibians (Lodé, 1996; Eggert, Peyret & Guyétant, 1999).

We used fluorescent pigment for indirect visual tracking and telemetric studies with implantable transmitters on the spadefoot toad (*Pelobates fuscus*) to gain information on habitat use by this secretive, fossorial and nocturnal toad. The present paper describes these two tracking procedures, and also reviews their advantages and limitations for obtaining movement information critical for population management and conservation.

MATERIALS AND METHODS

Five adult toads (two males aged 2 and 3 years and three females aged 4, 7 and >2 years) were caught between 6 and 25 May, 1998 while leaving two natural breeding ponds in north-eastern France (see Eggert & Guyétant, 1999 for site description and age estimation method). The transmitter implantations were done in the laboratory under general anaesthesia with 2-phenoxyethanol, an anaesthetic used for fish (Deacon, White & Hecht, 1997). Approximately 0.1-0.2 ml phenoxyethanol was mixed in 100 ml water and the toads were immersed until muscular relaxation was observed (30-40 minutes). After making a small incision mid-laterally in the left flank of each toad, the transmitter (BD-2GH, Holohil Systems, Ontario, Canada) was placed in the body cavity. Sutures of 6-0 polypropylene thread were made before washing the toad in running tap water. The animals recovered after 10 to 30 mins and, after observation for two days to verify full recovery, were released in the field at their exact places of capture. The implant volume (16 x 9 x 8 mm) represented about 10% of the normal abdominal volume of eggs, and its mass (2 g) was 9 to 11% of the body mass.

Animals were located in their burrows every day until 18 July (i.e. 52 to 71 days) using classical local triangulation methods and recorded to a precision of 10 cm, using a compass and a measuring tape. Searches for active, radio-tracked and non-radio-tracked toads were made over 34 nights. Every captured toad was tagged with a Passive Integrated Transponder (PIT tag) for individual identification.

Orange and yellow fluorescent pigments (Radiant Color, Ltd) were used with four of the radio-tracked toads and with 37 other, non-radio-tracked adults found active at night in the same area, so that the availability of habitat types could be considered the same for all toads. The dye was diluted in paraffin oil and applied in the field with a brush to the undersides of the four legs of the toads. We did not dig up any toads. As *P. fuscus* burrows daily, the colour pigments were removed by burrowing. During the following night we used a 6 W UV lamp to locate the pigment that had rubbed off onto

the ground or vegetation. We marked the path with a blue, fluorescent spray, visible under normal daylight. Movement patterns were then reduced to a series of points, assessed with the same procedure as for radio-tracking data, and filmed using a video camera for further microhabitat analysis. To test habitat-type preferences, we estimated habitat availability in the central region of the toads' range by measuring the length of each vegetation structure encountered along randomly chosen lines. For this, four 50 m-long lines forming the branches of two randomly situated crosses were used. Then, differences between availability and use by toads were tested with *G*-tests for goodness-of-fit. A Mann-Whitney *U*-test was performed when data normality assumptions were not met. Sandy, open areas were made up of natural dune and small, newly-created areas cleared of vegetation.

Active toads were rarely found near a burrow, so almost all observed fluorescent trails were incomplete parts of the respective nocturnal excursions. Therefore, fluorescent trails were used to estimate diel activity areas of the radio-tracked toads in two ways: (1) with the two burrows used before and after nocturnal movement (located during the day with transmitters), or (2) with only the fluorescent trail.

Minimum convex polygons and 99% probability Jennrich-Turner bivariate normal ellipses were calculated using the Ranges V computer program (Kenward, 1990).

RESULTS

All five toads consumed food within 24 hr of anaesthesia, and recovered normal locomotory activity within this period. Although transmitters were all the same model, signal range varied from 25 to 60 m, depending on the unit. As toads never moved far in two successive days (maximum distance observed: 88.7 m), and all toads preferred to dig in previous burrow sites, field work did not suffer from this low signal range. Toads' burrows could rarely be recognized because toads take care to cover the entrance (e.g. Kuzmin, 1999) or because entrances were hidden *de facto* by surrounding vegetation. Classical local triangulation methods allowed the location of toads' burrows in, at worse, a half square metre area. Passive Integrated Transponders allowed confirmation of the exact location of the toads' burrows by scanning the half square metre with the PIT tag reader.

During the period of the study, each toad used five to eight burrows and moved from one site to another between five and 15 times. Thus, the same hole could be used again several days after desertion (up to 11 days). One toad burrowed in the same place on 37 consecutive days. We observed that not all individuals emerged every night. A five-day period of inactivity was often observed in both sexes. The shortest distance between two successive burrows was 0.2 m and the longest was 88.7 m. The mean distance moved between two successive burrows was higher in females (22.9 m, SD=13.0
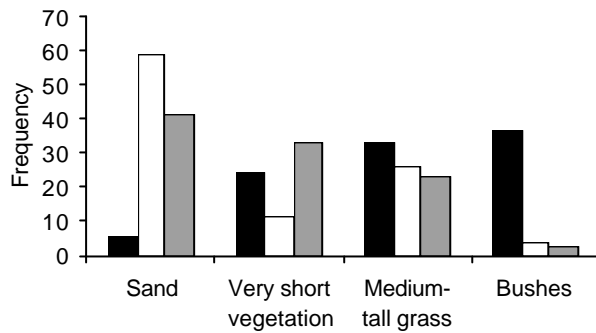
FIG. 1. Proportions of terrestrial habitat in four categories available in the field (black bars) and proportions of distance travelled by toads in each category (open bar: radio-tracked toads; striped bar: non-radio-tracked toads).

m, n=23) than in males (12.9 m, SD=19.6 m, n=12) (Mann-Whitney U-test, U=62, P<0.008).

Relocating the same toad on subsequent nights would have been almost impossible without radio-tracking, mainly because of the very cryptic coloration pattern. As a result, home-range would have been difficult to estimate.

Orange pigment was the most visible colour under ultraviolet light. The nocturnal trail was detectable along its entire course for the most part, although greater care was needed to find the fluorescent grains after a trail exceeded 15 to 25 m. The maximum distance recorded was 73 m, in a smooth, sandy area, but crossing vegetation (grass, moor) reduced the maximum trail length. No toads moved exclusively in vegetation during a night.

Detection of the trail was possible in all kinds of terrestrial habitat, even when wet. Pigments persisted on the ground and the vegetation for several days, even after light showers – this could be distracting when individuals moved around the same area several times. However, the blue fluorescent dye used to display the trails enabled us to distinguish individual trails, and it was possible to display all the trails in one area until the first heavy rain.

For radio-tracked and non-radio-tracked toads a significant difference between habitat availability and habitat use was observed (Fig. 1; G-tests for goodness-of-fit, respectively G=1833, df=3, P<0.0001 and G=2231, P<0.0001). Distributions of habitat use between the two categories of toads were distinct (χ^2=39, df=3, P<0.01) because of the different use of sandy areas and areas with very short vegetation. The greater use

of sandy areas by radio-tracked toads was mainly due to two individuals that, when first crossing a small, newly-created sandy area, kept within this area more precisely. No other toads ever crossed this test area. Overall, sandy places were clearly attractive, whereas shrub-covered areas were avoided by toads both with and without transmitters.

The vertical components of movements were obvious, particularly in grass – in dense, tall grass movements occurred throughout the vegetation (about 5 to 10 cm high), whereas in sparsely vegetated areas the toads moved on the ground.

Because of daily homing behaviour, it was more relevant to calculate diel home range than total activity area (Mullican, 1988). As there were no significant differences in diel home range between all radio-tracked individuals (Mann-Whitney test P>0.05), we pooled data from these toads to compare estimates of mean diel activity area obtained with pigment alone and with pigment and burrow information (Table 1).

Estimates of diel home-range were larger when radio-tracking data were included (P=0.06, Table 1).

DISCUSSION

PIGMENTS

Fluorescent pigments gave information similar to continuous radio-tracking methods (but without a time component) and gave better spatial accuracy, and so were better for estimating the diel home-range (Mullican, 1988). The pigment method is inexpensive and easy to set up. It could be used on many individuals as long as their trails could be distinguished from one another. This method can be used to study dispersal (Gibbs, 1998; Seabrook & Dettmann, 1996) as, for example, at the post-metamorphic stage (Demaynadier & Hunter, 1999; Sinsch, 1997). Edge effects, corridor use and foraging strategy might be inferred with increased precision. A possible drawback is that stress from handling might affect the behaviour of studied individuals, as reported for lizards (Dodd, 1992). However, *Pelobates fuscus* are placid and were not obviously disturbed by short handling periods of 10 to 30 seconds. Regarding physical stress on animals and impact on locomotion or burrowing behaviour, both will be lower with fluorescent pigments than with Dole's (1965) trailing device. Moreover, pigments could be used with very small individuals.

TABLE 1. Estimated daily activity area determined by fluorescent pigments for 14 diel trails of radio-tracked toads, with and without the location of the starting and ending burrows. The burrows were located using telemetry. Statistical comparisons used the paired t-test.

Method	Pigments + telemetry (m²)			Pigments only (m²)			P
	Mean	Range	SD	Mean	Range	SD	
Minimum convex polygon	23.5	2.2-98	28.4	16.4	2.1-80	20.4	0.06
Ellipse	148.2	11.4-572.9	183.1	105.24	12.2-379	112.4	0.11

The vertical component of habitat use is seldom considered in amphibian microhabitat studies. Fluorescent pigments provided useful data on this aspect, and as we diluted the powder in paraffin oil, the persistence of tracks was better under dew or in the wind than pigments used alone (pers. obs.). Moreover, unlike other methods (Mullican, 1988), the technique worked equally well on bare ground and in thick vegetation, except that plant cover reduced the detectable trail length. Fluorescent dye is especially appropriate for most species of amphibian because of their relatively short daily movements. *Pelobates fuscus* is a 4-7 cm long walking species with thin skin. Thus, we applied only small amounts of coloured paraffin oil. With other species that are larger, or that rely more on jumping, it should be possible to improve the maximum length of trail detection by using more paraffin oil and dye.

IMPLANTABLE TRANSMITTERS

Implantable transmitters appear more suitable than external ones, especially in fossorial species, as they do not injure the animals when they burrow into the ground. As observed in other studies (Madison, 1997; Madison & Farrand, 1998; Olders *et al.*, 1985) the implants seemed not to influence behaviour. Individuals with implanted transmitters were easily located, allowing observation of temporal and spatial activity patterns without artefacts resulting from troublesome harnesses (Golay, 1996). Similar implantation procedures have proven effective (Colberg *et al.,* 1997; Madison, 1997; Older *et al.*, 1985; Sinsch, 1988, 1991; Spieler & Linsenmair, 1998; Werner, 1991). The main limitation of telemetry with implantable transmitters, besides transmitter size and battery duration, is the quite low signal range. This restriction could became problematic with very mobile species, for example during migration (van Gelder *et al.*,1986*a*; Sinsch, 1990). Signal range will probably increase with the coming improvement of small batteries.

COMBINED USE OF TRANSMITTERS AND PIGMENTS

The combined use of implantable transmitters and fluorescent pigments in amphibian movement studies allows additional observations that cannot be obtained with either method alone. Without telemetry it would be difficult to relocate cryptic animals such as spadefoot toads, and thus to reapply fluorescent dyes on successive days. In the case of daily homing behaviour or movements in patchy environments, radio-tracking was informative for estimating home range or activity area only with intensive relocation efforts. Usually, this disturbs the animal, changing its movement patterns, and reduces the reliability of the results (Mullican, 1988). This did not happen with the pigment method because of the absence of an observer during animal movements. Pigments allowed us to get more data points and more information on use of microhabitats and burrowing sites, with quality-control.

As a general rule, cryptic toads were much more easily discovered in open areas than in tall grass or shrub-covered areas. Consequently, the occurrence in vegetation of toads without transmitters is more likely to have been underestimated than the occurrence in vegetation of radio-tracked toads.

SPADEFOOT TOAD HABITAT USE AND CONSERVATION IMPLICATIONS

Toads strongly preferred areas of bare ground or low vegetation. Shrub-covered places were avoided. We even observed that a toad that passed directly beneath a copse of willows without leaves during its migration to a breeding pond in April, stopped five meters in front of the same, leafy bushes after breeding in early June. In the end, the toad retreated about 50 m and went round this 400 m² copse. We could therefore assume that visual signals play a major role in such behaviour.

It is well known that spadefoot distribution is restricted locally to specific areas with a friable soil texture, because of the fossorial behaviour of the species (e.g. Nöllert, 1990; Kuzmin, 1999). River banks with alluvial sand deposits should be the most suitable spadefoot toad habitat according to Meissner's (1970) soil choice experiments. Our observations suggest that dense copse can inhibit adult spadefoot toads' dispersal. This was in agreement with Kauri's (1946) observations concerning the species' range expansion following deforestation by farmers. Nevertheless, spadefoot toads can be found in open forest (Kuzmin, 1999). As dense, shrubby vegetation represents the natural succession of vegetation in our studied area, population management measures in progress focus greatly on controlling vegetational succession.

Diel home range and microhabitat use can be better defined with the combined use of telemetry and pigments. These two methods can give relevant information about movement patterns that are required for habitat restoration and population management.

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