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SHORT-COMMUNICATION

Tracking a small cryptic amphibian with fluorescent powders

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Abstract: The study of amphibian spatial behaviour provides key information for species conservation. Most commonly used techniques to track amphibians are either unsuitable for small species or fail to give sufficiently fine-resolution data of habitat use. We report on the use of non-toxic fluorescent powders to track the fine-scale movement of a threatened New Zealand frog, *Leiopelma pakeka*. We assess the effect of powder application on frog movements, detection of frog pathways during a dry and a wet period, and the use of this marking technique after a translocation for conservation purposes. Our results show that fluorescent powders can be successfully used to obtain detailed information of fine-scale movements and habitat use of frogs, even during rainy periods. All frogs remained alive throughout the study period and no ill effects were noticeable. This technique has potential use for tracking other species that are too small or cryptic to be tracked using more conventional methods.

Keywords: frog; *Leiopelma pakeka*; movement; New Zealand; non-toxic fluorescent powders

Introduction

Information on amphibian spatial behaviour is crucial for a better understanding of species ecology (Duellman & Trueb 1994; Pittman et al. 2014) and for conservation purposes where key habitat features aid in management (Eggert 2002; Lemckert 2004). Various techniques are available to track amphibians; however, their use is often limited by the size of the focal species and the method of tracking device attachment. Small (<7 g; Rowley & Alford 2007) and burrowing species represent a challenge as commonly used techniques, such as radio-telemetry, are too heavy, may injure the animal when burrowing (Eggert 2002; Graeter & Rothermel 2007; Rowley & Alford 2007), or are not appropriate for detecting fine-resolution habitat use (Lövei et al. 1997; Birchfield & Deters 2005). Non-toxic fluorescent powders have been used to track small animals including insects (e.g. Johansson 1959; Vardeman et al. 2007), mammals (e.g. Lemen & Freeman 1985; Mullican 1988), and amphibians (e.g. Woolbright 1985; Birchfield & Deters 2005; Ramirez et al. 2012). This tracking method has proven harmless for amphibians (Rittenhouse et al. 2006; Orlofske et al. 2009) while providing detailed data on small-scale movements and habitat use (Eggert 2002; Graeter & Rothermel 2007).

Here we report the effectiveness of non-toxic fluorescent powders to track fine-scale movements of the native New Zealand frog, *Leiopelma pakeka*. We evaluate (1) the effect of the powder on the frogs' movement behaviour, (2) the effect of weather on the detectability of path length, and (3) the use of this technique for monitoring translocated individuals for conservation purposes.

Leiopelma pakeka is a small, terrestrial, cryptically-coloured and nocturnal species with the only naturally occurring population found on Maud Island, Marlborough Sounds (Bell 1978; Bell & Pledger 2010; Bishop et al. 2014). It is one of the largest extant *Leiopelma* species, with snout-vent length of females greater than 40 mm and 34–40 mm in males or young females (Bell 1978; Newman 1990; Bell et al. 2004). Adults are highly sedentary with individuals occupying discrete home ranges of 26.7 ± 2.2 m^2 (Bell 1994; Bell et al. 2004; Webster 2004) over a period of decades (Bell & Moore 2015). The species is considered to be 'vulnerable' both at a national and international level (Newman et al. 2013; IUCN 2015). To date, studies of *L. pakeka* have focused on describing patterns of spatial distribution within small long-term study plots (12 × 12 m plots studied since 1983), relying heavily on mark-recapture techniques (e.g. Newman 1990; Bell 1994; Bell et al. 2004; Webster 2004; Germano 2006; Bell & Pledger 2010). However, the information obtained at an individual level is often limited by a single nightly record of capture locations over a limited capture period. Moreover, it depends on the recapture rate and survey area so the space and time accuracy is usually quite coarse (Lövei et al. 1997; Eggert 2002). To improve the conservation status of *L. pakeka*, several translocations have been carried out since 1984 (Bell 1994, 2010). Homing tendencies are one of the biggest problems affecting translocations (Matthews 2003; Sullivan et al. 2004; Tocher & Brown 2004). Therefore, being able to track post-translocation movements can help us to understand the behaviour that impacts translocation successes.

Methods

Powder application and path marking

Frogs used in this study were caught opportunistically on Maud Island (41°01'S, 173°53'E), Marlborough Sounds, within a 16 ha remnant of broad-leaved forest (described in

Bell & Bell 1994). Once a frog was detected on the ground, it was captured by hand, measured (snout-vent length) and photographed. Frogs had non-toxic fluorescent powders (ECO-Series, Dayglo Color Corp, Cleveland, USA, colours: green, yellow and magenta; or R-105 Series, Radiant Color Ltd., Houthalen, Belgium, colours: green, yellow, blue and magenta) applied to their bodies in the field by placing the frog on top of the powder to cover the ventral surface and legs, ensuring it would stick to their feet. After measurements and powder application (handling time <1 minute), frogs were immediately released at their capture location (or release site) and researchers departed to ensure minimal disturbance. After every 30 minutes, the pigmented trail left behind by the frogs as they moved was checked using a portable UV light (MTE UV301, Urban Outback Gear, Wallsend, Australia). At each time interval (i.e. every 30 minutes), we marked the location and the change of direction (turn) relative to the previous mark with either wooden pegs the size of a toothpick or cloth tape (Fig. 2).

Effect of handling and powder application on frogs' movement

To investigate the effects of the powder application and handling of frogs on their movements, in March 2014 five randomly selected frogs were tracked using a night vision scope (Yukon NVMT 3 (4x50) Prowler Night Vision Monocular, Vilnius, Lithuania), without any type of handling or powder. Frogs were tracked for two continuous hours each and after every 30 minutes observation a mark was placed on the frogs' pathways as described above. To establish the impact on the frogs' movement behaviour, we compared the total distances moved during the first 2 hours among 30 powdered and these five non-powdered frogs (measured the following day as a straight-line between successive marks).

Effect of weather on path detectability

To investigate the effect of weather on the detectability of frogs' pathways, 30 randomly selected adult frogs were tracked using fluorescent powders for one night each during a dry period (December 2014, no rain during the five tracking nights) and another 30 during a wet period (April 2015, rain during all three tracking nights). Frogs were tracked throughout the night to ensure their wellbeing and to obtain information of their entire activity period. Of the 60 tracked frogs, 82% were females and 18% males. The mean precipitation during the tracking nights in the wet period was 27.4 mm, whereas there was no rain in the dry period. The path length (i.e. total distance moved) was recorded the following day as described above. Tracking period, total time (hours) spent tracking frogs during a night until all frogs sought a final retreat site, was also recorded. A retreat site was considered as 'final' when a frog went inside after dawn, or during the night but stayed inside until after dawn.

Use of powder for monitoring translocated frogs

Fluorescent powders were used to investigate frogs' pathways and dispersal following a translocation. Frogs (n = 101) were translocated in July 2005 from Maud Island to Long Island (41°07'S, 174°17'E), Queen Charlotte Sound, in a release site of 10 × 12 m dominated by broadleaf tree species (Germano 2006). Twenty-five of the released frogs were tracked using fluorescent powders during the first night following release. After the release, frogs were tracked every 30 minutes until they found initial retreats. Total distances moved were recorded the following day as described above, as was the compass bearing from the release point to the path end.

Statistical analyses

Because assumptions of parametric tests were not met due to the small sample sizes and unbalanced design (non-powdered vs powdered frogs), linear data were analysed using non-parametric Kruskal-Wallis tests. Rayleigh's test for uniformity was used to determine if bearings were uniformly distributed. Analyses were performed in R version 3.2.0 (R core Team 2015) and Oriana (version 2.0, Kovach Computing Services). Summary statistics presented are means ± 1 SE.

Results

Effect of fluorescent powders on frogs' movement

All frogs remained alive throughout the study periods and were not obviously disturbed by the handling or powder application. The powder remained on the frogs and left a noticeable trail during the entire night so there was no need for re-handling or re-application, and the different powder colours did not affect the detectability of the paths. All five non-powdered frogs fell within the distribution of the powdered frogs when plotted against the total distance moved (Fig. 1). The mean total distances moved by the five non-powdered frogs did not differ significantly from the mean total distances moved by the 30 frogs tracked with powders (1.12 ± 0.26 m vs 1.56 ± 0.23 m, respectively; H = 0.18, P = 0.67).

Effect of weather on path detectability

During rainy nights, trails were less noticeable and faded faster than during dry nights but it was still possible to detect frogs' pathways. The mean tracking period was significantly longer during the wet period compared to the dry period (8.68 ± 0.12 h vs 6.92 ± 0.15 h per night; H = 37.52, P < 0.001). Although frogs were tracked for a similar amount of time during each night, some frogs retreated into their final retreat sites before midnight whereas others retreated after sunrise. Mean path length did not differ between the dry and wet periods (H = 0.34, P = 0.559; Table 1).

Use of powder for monitoring translocated frogs

Of the 25 tracked frogs, 21 left tracks to retreats. Four trails ended when the powder became too faint to follow. The mean path length was 1.31 m ± 0.25. The mean bearing for these paths was 309.3° ± 16.3° and bearings were not randomly distributed (Z = 5.45, P = 0.003).

Discussion

Due to the relatively small size of *L. pakeka* and the fact that a large proportion of its life is spent under large rock piles, obtaining detailed information on individuals' movements and habitat use can be difficult as fine-scale tracking methods (e.g. harmonic radar tracking) are quite limited for small species. Germano (2006) assessed the homing abilities of *L. pakeka* individuals displaced from their home range using harmonic radar tracking. However, as with traditional radio telemetry studies, its precision relies on the number of relocation points

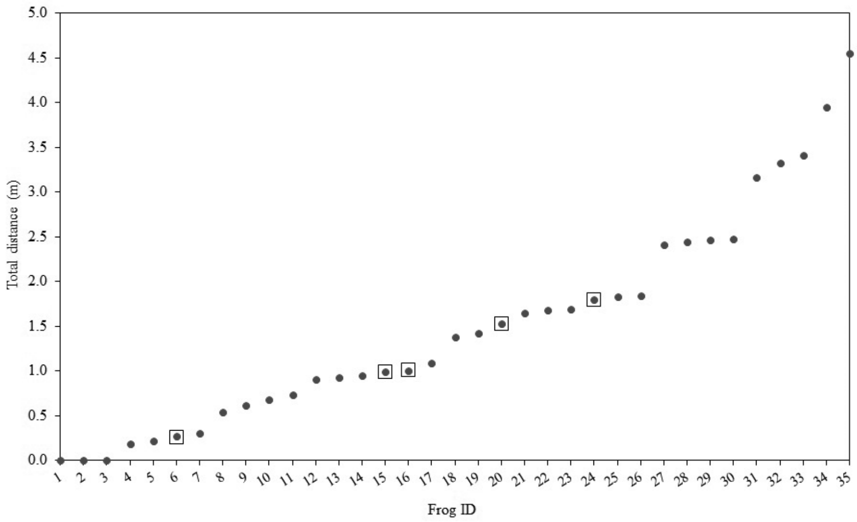

Figure 1. Total distances moved during the first two hours of tracking frogs with fluorescent powders (*n* = 30) and with night vision scope (*n* = 5; marked with squares).

Figure 2. Frogs' post-translocation movements shown as (a) three fluorescent trails marked with red cloth tapes after 30 minutes of observation/turn and (b) with white lines highlighting the pathways (photos by JM Germano).

Table 1. Measured path lengths (i.e. total distance) for frogs tracked with fluorescent powders during a dry (December 2014) and a wet (April 2015) period. Mean path lengths did not significantly differ between periods (H = 0.34, P = 0.559).

Period	Number of tracked frogs	Mean precipitation during tracking nights (mm)	Path length (m)		
			Minimum	Maximum	Mean ± SE
Dry	30	0	0.34	12.93	5.13 ± 0.57
Wet	30	27.4	0.75	12.44	4.72 ± 0.56

and it does not give a detailed description of movement or habitat use. Furthermore, while harmonic radar tracking can be used for small species (Langkilde & Alford 2002), it does not allow for individual identification without extra manipulation. Despite testing on captive frogs, a small proportion (2 of 11 frogs) of the wild *L. pakeka* tracked using harmonic radar died due to prolonged excessive muscular activity, with necropsy reports attributing this to capture myopathy or exertional rhabdomyolysis (Germano 2006). This mortality rate suggests that other less intrusive techniques, such as fluorescent powder tracking, may be more appropriate and safer to use with threatened Leiopelmatid frogs.

This is the first evaluation of the use of fluorescent powders as a tracking technique for *Leiopelma*. The use of these powders for tracking frogs was quite efficient. Detailed information on the fine-scale movements of frogs was obtained without much disturbance. Powders were quickly and easily applied involving minimal handling of frogs, with a handling time that is less compared to other tracking techniques as no tracking device is either attached or inserted. We detected no evidence of negative effects on frogs, such as death or unusual behaviour (e.g. attempts to remove powder) and there was no need for re-handling or for re-application of powders, even during rainy nights. Where fluorescent powders have been used previously, they were still visible on frogs two days after first application in the absence of rain (Rittenhouse et al. 2006). In our study, some frogs could be seen with residues of powder on their bodies the following night but considerably less than when first applied, and the majority of them no longer left a trail. Rain speeded up the removal of powders from the frogs' bodies and habitat; subsequently, from December 2014 to April 2015 only some areas had visible remnants of powder.

Some studies have tested the detectability of different powder colours (Birchfield & Deters 2005; Graeter & Rothermel 2007), which helped us in the selection of colours for our study. The colours we chose (yellow, green, blue, and magenta) allowed us to track the movement of frogs until they reached their final retreat sites, even during wet periods. Yellow and green can be difficult to differentiate under UV light, nonetheless, using both or either of these along with magenta and blue allowed tracking of adjacent animals and differentiation between individuals. Additionally, although it was not tested here, powders were detectable in all the different microhabitat types present in the area, including on wet vegetation and on trees.

Handling and releasing marked individuals can be problematic as it can affect their behaviour, therefore attempts should be made to measure any negative effects of the marking technique (Turchin 1998). As measured by total distances moved, we found no major influences of the use of fluorescent powders for tracking frogs' movements. Mean total distances travelled by powdered frogs were not significantly different from the distances travelled by frogs tracked with night-vision equipment. While both groups (powdered and tracked) were potentially influenced by researcher intervention, we conclude that powders can give accurate information on frogs' movements despite the initial manipulation needed to apply the powders, the release method and the 30 minute checking intervals.

Rain did not affect the detectability of the frogs' pathways. During the dry and wet periods, frogs were tracked during the entire night and the detected mean path lengths did not differ significantly. By allowing enough powder to cover the legs and ventral skin of the frogs tracked during the wet period, it was possible to detect the frogs' pathways during rainy nights. Furthermore, because nights are longer during the wet season, the tracking period was longer and yet the powder remained on the frogs long enough to track their entire movement. Most amphibian studies check the trails left by individuals after a few hours or even after 24 hours (e.g. Graeter & Rothermel 2007; Ramirez et al. 2012; Pittman & Semlitsch 2013), but this time interval can present disadvantages as paths can be confused by trails crossing, heavy downpours erasing trails, old and new trails being confused as the powders can remain visible for 1–2 days in absence of rain (Graeter & Rothermel 2007; P. Ramirez, pers. obs.) and paths becoming faint as distance from release site increases not allowing clear identification. By tracking frogs every 30 minutes throughout the night we were able to detect a clear fluorescent trail allowing a more accurate description of frog movements during their activity period and during two different weather conditions.

Translocations are increasingly common in wildlife management but their effectiveness can be reduced by the homing instinct of many species, so it is important to examine how species respond to translocations. Fluorescent powders proved useful as they rendered a very detailed description of the paths taken by frogs immediately upon release. Paths had a mean bearing NW (309.3°) which is close to the NW (320°) bearing from Maud Island (the capture site). This pattern could reflect a homing inclination towards their capture site but since the ability to home is negatively correlated with the displacement distance (Sinsch 1991; Gonser & Woolbright 1995), it is unlikely that an immediate homing instinct would be present as this translocation took place roughly 25 km from Maud Island. A more plausible explanation for this directional movement upon release is the availability of better habitat quality uphill in a W–NW direction (Germano 2006).

Given that *L. pakeka* is a threatened species, future studies could further evaluate the effect of the powders on its physiology, reproduction and health. Additionally, because individual frogs were tracked for one night only, we cannot establish the effect powders may have on subsequent nights, therefore, the possible effects of prolonged exposure (i.e. more than one night) on frogs still needs to be assessed. This technique could also be useful for tracking other species from different taxa which are too small or cryptic to be tracked with conventional methods.

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