
A FIRST TEST OF THE THREAD BOBBIN TRACKING TECHNIQUE AS A METHOD FOR STUDYING THE ECOLOGY OF HERPETOFAUNA IN A TROPICAL RAINFOREST

EMILY WADDELL^{1,2,3}, ANDREW WHITWORTH^{1,2}, AND ROSS MACLEOD²

¹The Crees Foundation, 7-8 Kendrick Mews, London, SW7 3HG, UK and Urbanizacion Mariscal Gamarra B-5, Zona 1, Cusco, Peru

²Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK

³Corresponding author, e-mail: emilyhelen22@hotmail.com

Abstract.—The lack of information about amphibians and reptiles in highly threatened tropical rainforest habitats has led to a need for innovative methods that can rapidly generate data on ecological behavior. The thread bobbin technique has proven successful for gathering ecological information in a range of habitats, but has not yet been used in tropical rainforests. Here we test the method for the first time in a humid tropical forest habitat on 14 herpetofaunal species. We found thread bobbins to be effective for large anurans (one leptodactylid and one bufonid), medium-large terrestrial snakes (one booid, three colubrids and one viperid), and testudines (one chelid), but largely unsuccessful for arboreal snakes (one booid and one colubrid), small and slender snakes (two colubrids), and small anurans (one strabomantid). We tracked 18 individuals for 1.2–15 d (mean 4.6 d) for distances of 5.5–469.3 m (mean 159.2 m). The thread trail revealed the exact movements of the tracked animal, providing detailed information on activity and microhabitat use that many alternative tracking methods cannot provide. Conservation projects rely heavily upon understanding the life history of species and without this prior knowledge, conservation efforts can fail, wasting funds and resources. We show that the thread bobbin method is a cost-effective technique that can be used to rapidly gather detailed ecological information on the life history of relatively unknown rainforest reptiles and amphibians.

Key Words.—activity; amphibians; life-history traits; microhabitat use; rapid ecological surveys; reptiles

INTRODUCTION

Amphibians and reptiles are key components of their ecosystems (Heyer et al. 1994; Beaupre and Douglas 2009; Hillman et al. 2009; Foster et al. 2012), yet both groups are threatened worldwide. Declines are steepest in the most diverse regions of the world such as tropical rainforests (Duellman 1999), due to an amalgamation of factors including habitat destruction, invasive species, exploitation, climate change, and disease (Lips 1998; Gibbons et al. 2000; Collins and Storfer 2003; Stuart et al. 2004). These threats are likely underestimated due to the lack of basic ecological knowledge of rainforest amphibians and reptiles. As a result, true distributions and population trends remain undetermined. For example, 25% of evaluated amphibians and 18.3% of reptiles are classified as Data Deficient by the IUCN Red List (IUCN. 2015. The IUCN Red List of Threatened Species. Version 2015.2. Available from <http://www.iucnredlist.org> [Accessed 17 October 15]). This is emphasized by the low numbers of reptiles that have been evaluated by the IUCN; just 43% of known species compared with almost all known species of birds and mammals and 86.1% of amphibians (IUCN. 2015. *op. cit.*). The lack of ecological information on rainforest

herpetofauna may be partially attributed to the challenges of surveying in this dense habitat and often difficult terrain. Thus, there is a need for innovative survey methods that can be used to help gather ecological data on herpetofaunal groups (Böhm et al. 2013).

The most basic and frequently used method to study the ecology and habitat preferences of tropical rainforest species involves the collection of descriptive data from simple field surveys (Heyer et al. 1994; Duellman 2005; McDiarmid et al. 2012; Beirne et al. 2013). This method can contribute important ecological knowledge, but is generally limited to providing single data points for individuals. In contrast, tracking methods can generate large amounts of detailed ecological data by the repeat location of target individuals over several days (Heyer et al. 1994) and can be used to investigate home ranges, dispersal, activity patterns, habitat preferences, and microhabitat use. External and internal radio transmitters are the primary method for animal tracking and have been successfully used on a wide variety of herpetofauna in a range of habitats including tropical rainforest (Eggert 2002; Kay 2004; Rowley and Alford 2007; Wasko and Sasa 2009). More recently, automated telemetry has also been used to track a range of

rainforest species (Kays et al. 2011), overcoming many of the shortfalls of traditional radio tracking in this habitat.

A less conventional method to study ecology and habitat preferences is the use of radioactive isotopes, in which a device with small amounts of radioactive material is implanted inside an animal (Ashton 1994). Radioactive isotopes have been successfully used on both amphibians and lizards (Munger 1984; Thompson 1993), although this method is no longer widely used due to welfare concerns and difficulties with licenses (Beausoleil et al. 2004; Mellor et al. 2004). The smaller the device for both methods, the lower the detectability (Munger 1984; Mellor et al. 2004), which is decreased further in dense vegetation and can, therefore, be a major limitation within tropical rainforest habitat (Cresswell 2005). The biggest disadvantage of these methods is that they only allow data to be gathered when an individual is relocated, and thus distances are measured along the straight line between relocations and habitat preference information is limited to relocation sites only. Furthermore, some of these methods are expensive and require high levels of expertise for internal implants, which are also highly intrusive.

Novel or less conventional techniques have also been developed to provide detailed information on movement patterns and microhabitat preference that are not possible using conventional methods, resulting in the ability to collect more ecological data over a shorter period of time (Tozetti and Martins 2007). The fluorescent powder technique involves covering the ventral surface of an animal with UV powder so that UV traces are left on the substrate as the individual moves, which can then be followed using a black-light (Plummer and Ferner 2012). This method has been successfully used to study a range of herpetofauna in a variety of habitats (Blankenship et al. 1990; Eggert 2002; Stark et al. 2005; Rittenhouse et al. 2006; Furman et al. 2011). Another technique involves the external attachment of a thread bobbin via an adhesive so that the thread is pulled out as the animal moves, allowing the exact track of the animal to be recorded (Heyer 1994). This technique has been successful for several herpetofaunal species (Stickel 1950; Dole 1965; Díaz-Paniagua et al. 1995; Tozetti and Martins 2007). These methods are both relatively easy to use and cost effective (Mellor et al. 2004). However, fluorescent powders have limited success in tropical rainforest habitat, providing a maximum total tracking distance of just 16.65 m for amphibians (Lindquist et al. 2007) and 60 m for small mammals (Nicolas and Colyn 2007). The thread bobbin method has yet to be tested in rainforest habitat, but tracking distances of up to 300 m in semi-humid tropical grass and shrublands (Tozetti and Toldeo 2005; Tozetti and Martins 2007) indicate that the thread bobbin method has the potential to be successfully used in tropical rainforest habitat to gather

information over a greater distance than that of UV powders.

This study tests for the first time the thread bobbin method on a variety of herpetofaunal species in a tropical rainforest to find out which reptile and amphibian species and groups can be successfully equipped with a thread bobbin device. More specifically, we evaluated the longevity of bobbins as tracking devices and report the tracking distances of different reptiles and amphibians. A final objective of our research was to compare the thread bobbin technique to other tracking methods in terms of cost, effort, and the type of information collected.

MATERIALS AND METHODS

Study site.—We conducted field research between 2 July and 4 September 2012 at the Manu Learning Center (MLC), in the Manu Biosphere Reserve, southeast Peru. The MLC is a research station within the Fundo Mascoitania (12°47'21.9"S, 071°23'30.5"W), a 650 ha reserve operated and managed by the Crees Foundation. The reserve is located in regenerating tropical lowland rainforest in the Amazon basin to the east of the Andean foothills with an elevation ranging between 450–740 m (for a detailed description of the study site, see Whitworth et al. 2016).

Attachment methods.—We captured all animals opportunistically or during visual encounter surveys as part of the research and monitoring program of the Crees Foundation. We brought back each individual to the MLC to accurately measure the body mass and length, only attaching the bobbin to individuals with a body mass of 70 g or more so that the device represented no more than 10% of the overall body mass of an animal, as recommended by Richards et al. (1994) for short term attachment. However, in most cases it was well below 10%. We used a nylon thread cocoon bobbin (Danfield Ltd., Leigh, UK; Fig. 1a), which unwound from the inside out and came in two strengths: normal and double strength. Each bobbin was 39 mm in length, 14 mm at the widest part and tapered towards each end. The weight was 4.5 g per full bobbin and the thread was a total length of 500 m for normal strength and 250 m for double strength. We used half bobbins on individuals close to the 70 g weight minimum or particularly slender snake species. We created half bobbins by manually extracting thread until the weight of the bobbin was halved. Before attachment, we enclosed the bobbin in plastic wrapping (cling film) with a small hole at one end to allow the thread to unwind. This ensured that none of the thread was stuck to the adhesive and the animal would be left unattached to the thread once it finished. For snakes, we attached bobbins to the dorsal lateral region at the posterior third of the body using duct

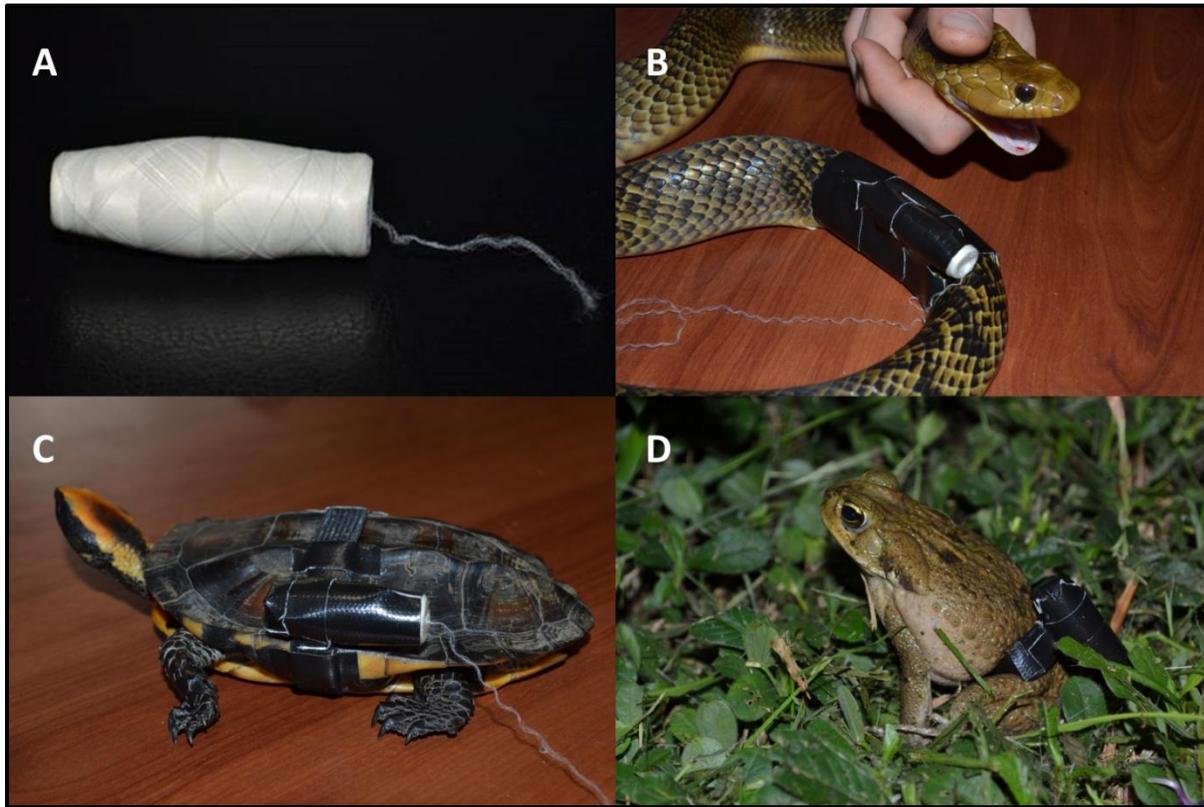


FIGURE 1. Nylon cocoon bobbin (A), attachment for snakes (B), turtles (C), and anurans (D). (Photographed by Emily Waddell).

tape (Gorilla Tape[®], The Gorilla Glue Company, Cincinnati, Ohio, USA; Fig. 1b). We chose the amount of tape used based upon the size of the individual, though the tape was always attached halfway around the girth of the body avoiding the ventral scales so that the device would not restrict internal functions. Before using the tape, we rounded the corners to decrease the chances of the tape peeling loose when the animal moved through the substrate. We attached the bobbin via a black elastic harness around the waist/carapace in anurans and turtles (Fig. 1c and 1d). To fit the harness, we measured the waist/carapace of each individual and cut the elastic (6 mm wide) to this measurement. We secured the ends of the elastic with two small pieces of duct tape across and around the join. We covered each bobbin in duct tape and attached them to the harness using a thin strip of duct tape secured by a further two smaller strips of tape. We used only normal strength bobbins for this attachment.

We tested the tracking potential of thread for smaller medium-bodied anuran species (weight < 70 g) and very slender snake species by exploring a thread-end attachment strategy. We secured the bobbin to the habitat and attached the thread end to the animal via a small harness for anurans or directly using super glue and a small piece of duct tape for snakes. We released

these individuals within a controlled area of the MLC gardens and observed how they moved. We used both strengths of bobbin when testing thread-end attachment.

Release and tracking.—We released animals at their capture site (marked using a Garmin eTrex H GPS, Garmin [Europe] Ltd., Southampton, UK, to an accuracy of 8 m) within 2 h of attachment and within 48 h of capture. We tied the loose end of the thread to something stationary within the habitat (e.g., the trunk of a tree or a branch) and the position marked with a yellow flag. We relocated each individual each evening (1600–2000) and morning (0600–1000) by following the thread from the last relocation site. At each relocation site, we marked the position of the animal with a yellow flag and recorded the GPS coordinates. We measured the length of the thread (equal to the effective distance moved; EDM) by laying a tape measure on top of the thread, starting approximately 2 m from the animal (exact position marked and measured once animal had moved) making sure not to disturb the animal. We measured the straight-line distance (SLD) between relocation sites by hand with a tape measure if the two locations were within sight of one another. When the relocations were too far apart to measure by hand, we calculated the SLD from the GPS points of the two relocations using Google

Earth (Google. 2013. Google Earth. Version 7. Available from <http://www.google.com/earth/download/ge/agree.html> [Accessed 10 July 13]). At each relocation, we recorded the activity and current microhabitat of the animal, which we recorded as one of five categories: (1) hidden in substrate; (2) exposed on substrate; (3) hidden in water; (4) exposed in water; and (5) in refuge. Additionally, we noted features along the thread trail: different substrates or microhabitats travelled through, minimum distance moved in water (to prevent overestimating the distance due to the potential of thread drag caused by water flow), distance spent off ground, and the maximum height. We tracked anurans for 3–5 d, depending on how delicate their skin was and based on recommendations by Dole (1965), and reptiles for as long as the method was successful (i.e., the bobbin started to come off or the thread ran out), which was up to 15 d. We attached a second bobbin to one anuran individual that had moved a large proportion of the thread length after 1 d. Where possible, we recaptured animals at the end of their tracking period (i.e., when the animals had not escaped due to the bobbin detaching or the thread running out) and removed the bobbin attachment, with care taken to remove duct tape from snakes through soaking in warm water to prevent damage to scales.

Technique analysis.—We assigned each individual to one of three distinct categories: Category 1 was an animal relocated more than twice and therefore deemed as having been successfully tracked; Category 2 was tracking data that was collected and was considered to be inadequate but had the bobbin successfully attached, and Category 3 was the method that was completely unsuccessful due to the method failing before relocation or the method could not be tested on the species as there was no way for the bobbin to be attached safely and ethically. We only used data from Category 1 tracking attempts to test the effectiveness of the method at gathering ecological data as adequate tracking data was needed for each individual to allow for sufficient comparisons to be made. This was done by comparing relocation only data and relocation plus thread trail data to quantify how much additional information the method provided.

Statistical analysis.—We carried out all statistical analyses in R 2.15.3 (R Core Team 2013). We tested all data for normality using a Shapiro-Wilk normality test and data were not normally distributed. Therefore, we analyzed data using the non-parametric Wilcoxon signed rank test. We compared the straight line distance (SLD) with effective distance moved (EDM) and relocation alone data with relocation plus thread trail data for the different substrates used and maximum height from ground.

Comparison of methods.—We compared the thread bobbin tracking for use in tropical forests to other tracking methods by categorizing specific variables into qualitative low, medium, and high categories. We compiled this using information primarily from method descriptions in Heyer et al. (1994), Beausoleil et al. (2004), and McDiarmid et al. (2012) as well as observations and conclusions from data collected within this study. We based equipment costs on prices of commonly used sources of equipment necessary to track one individual for the specific method and did not incorporate travel or labor costs. We categorized longevity as the range of time for which one individual can be tracked; our categories were low: < 3 mo (no seasonal dynamics captured), medium: 3–6 mo (some seasonal dynamics captured) and high: > 6 mo (seasonal to annual dynamics captured). Specific explanations for each category placement for both detail of data on activity and detail of data on microhabitat use (i.e., what part of the forest structure an animal moves through and use of refugia) are included in the table. The categorization of these variables take into account whether or not data can be collected in between relocations and how accurate is the relocation data. We categorized suitability over large distances as how suitable and practical the method is to track herpetofauna over a large area in a tropical rainforest; our categories were low if the method is not suitable, medium if the method can be suitable but data is limited, and high if the method is highly suitable for such studies. The Potential Impact includes the impact on the tracked animal other than being handled and ranges from low where the animal is subjected to the presence of the researcher during relocations to high where there is an invasive procedure as part of method. We recognize that other methods allow the measurement of more specialized variables, such as body temperature; however, we focused on the variables presented here as they are useful for carrying out basic ecological studies on poorly known species.

RESULTS

Test of bobbin method.—Overall, we tested the bobbin tracking method on 33 individuals of 14 species (Table 1). We collected detailed ecological data on 18 individuals (Table 2) of eight species that we successfully tracked (Category 1) with the bobbin attached either directly or via a harness. We tested the method on a further 10 individuals (from five species) for which data collected was considered inadequate (Category 2). We deemed the method unsuitable for a further five individuals (from four species; Category 3; Table 1).

We found that the thread bobbin method works well for large anurans (Cane Toad, *Rhinella marina*; Rose-

TABLE 1. Amphibian and reptile species on which the thread bobbin method was tested and the outcomes. Species abbreviations are RM = *Rhinella marina*, Lr = *Leptodactylus rhodomystax*, Oq = *Oreobates quixensis*, Ch = *Corallus hortulanus*, Ec = *Epicrates cenchria*, Ha = *Helicops angulatus*, La = *Leptodeira annulata*, Om = *Oxyrhopus melanogenys*, Op = *Oxyrhopus petolarius*, Ps = *Pseustes sulphureus*, Sc = *Siphlophis compressus*, Xs = *Xenodon severus*, Lm = *Lachesis muta*, Pp = *Platemys platycephala*. Abbreviations are M = Methods (B = bobbin only method, T = thread-end only method, and BH = both bobbin and thread-end method), C = Categories (1, relocated more than twice with adequate tracking data; 2, tracking data collected inadequate but bobbin successfully attached; and 3, method unsuccessful by failing before relocation or bobbin could not be attached), TS = tested successfully, HB = half bobbin used, BT = bobbin taken off, BF = bobbin fell off, TSA = thread snapped apart, TF = thread finished spooling, MR = movement restricted, L = animal lost, EH = animal escaped unharmed, and P = animal predated.

Species	M	C	No. of individuals				Tracking outcome					
			TS	HB	BT	BF	TSA	TF	MR	L	EH	P
Amphibians												
Toads												
<i>Rm</i>	B	1,2	14 (9)	2	10	0	1	2	0	0	0	1
Frogs												
<i>Lr</i>	B	1,2	4 (2)	2	2	0	0	0	0	0	2	0
<i>Oq</i>	T	3	1 (0)	-	0	0	1	0	0	0	0	0
Reptiles												
Boids												
<i>Ch</i>	B	2	1 (0)	0	0	0	0	0	0	1	0	0
<i>Ec</i>	B	1	1 (1)	0	0	1	0	0	0	0	0	0
Colubrids												
<i>Ha</i>	B	3	1 (0)	1	0	1	0	0	0	0	0	0
<i>La</i>	T	3	2 (0)	-	0	0	1	0	1	0	0	0
<i>Om</i>	B	1	1 (1)	0	0	1	0	0	0	0	0	0
<i>Op</i>	B	1	2 (2)	1	1	1	0	0	0	0	0	0
<i>Ps</i>	B	2	1 (0)	0	0	0	0	0	0	1	0	0
<i>Sc</i>	BH	3	1 (0)	1	0	0	1	0	1	0	0	0
<i>Xs</i>	B	1	1 (1)	0	0	0	1	0	0	0	0	0
Vipers												
<i>Lm</i>	B	1,2	2 (1)	0	0	2	0	0	0	0	0	0
Testudines												
<i>Pp</i>	B	1	1 (1)	0	0	0	0	1	0	0	0	0
Total			33 (18)	7	13	6	5	3	2	2	2	1

lipped Thin Toed Frog, *Leptodactylus rhodomystax*), medium to large terrestrial snakes (Rainbow Boa, *Epicrates cenchria*; Tschudi’s False Coral Snake, *Oxyrhopus melanogenys*; Forest Flame Snake, *Oxyrhopus petolarius*; Amazon False Fer-de-lance, *Xenodon severus*; South American Bushmaster, *Lachesis muta*) and a testudine (Twist-neck Turtle, *Platemys platycephala*), but was largely unsuccessful for arboreal snakes (Garden Tree Boa, *Corallus hortulanus*; Amazon Puffing Snake, *Pseustes sulphureus*), small slender snakes (Banded Cat-eyed Snake, *Leptodeira annulata*; Tropical Flat Snake, *Siphlophis compressus*), and small anurans (Common Big-headed Frog, *Oreobates quixensis*). We deemed the thread end attachment as unsuccessful after the thread readily snapped (standard thread) or restricted the animals’ movement (stronger thread). The bobbin was either removed by the researcher or it fell off at the end of tracking (once the thread snapped and once it finished) and no skin abrasions were recorded for any of the harness wearing individuals.

Effectiveness of thread bobbin method at gathering ecological data.—

We relocated 18 individuals (11 amphibians and seven reptiles) 167 times, with the animal stationary at 97% of relocations. We recorded the effective distance moved (EDM; median = 12.23 m, Inter-quartile range [IQR] = 48.9 m, n = 76), as indicated by the length of thread unwound between each relocation, which was more than twice the straight-line distance (SLD; median = 4.5 m, IQR = 16.98 m, n = 76) between relocations (Table 2). This difference was significant ($V = 3044.5$, $df = 333$, $P < 0.001$).

We investigated the number of different substrates used and the maximum height from the ground by comparing in relocation alone data (R hereafter) and relocation plus thread trail (T hereafter) data (Table 2). These differences were significant for both different substrates ($V = 0$, $df = 35$, $P = 0.001$) and maximum height ($V = 0$, $df = 35$, $P = 0.036$). We never relocated seven of the nine individuals that moved through water at least once in their tracking in water (Table 2);

TABLE 2. Comparison of ecological data gathered on activity, substrate use, and habitat use of amphibians and reptiles using thread bobbin method based on individual movement patterns recorded in tropical forest habitat. Abbreviations are: EDM = total effective distance moved, SLD = total straight-line distance between relocations, % of EDM = percentage the SLD is of the EDM, DSR = substrate data recorded solely at relocations, DST = substrate relocation data plus thread trail data, MHR = maximum height recorded solely at relocations, MHT = maximum height from relocation data plus thread trail data, MWR = number of relocations in water, and MWT = the percentage of the EDM that was in water. An asterisk (*) is an individual that had a second bobbin attached during tracking.

Individual	Tracked days (No. of relocations)	Total EDM	Total SLD	% of EDM						
					DSR	DST	MHR	MHT	MWR	MWT
<i>Rhinella marina 1</i>	4.5 (9)	465.6	154.6	33	3	4	0	0	0	5.6%
<i>R. marina 2</i>	5.0 (10)	367.5	178.0	48	2	3	1	1	0	1.6%
<i>R. marina 3</i>	5.0 (10)	128.5	55.55	43	2	2	0	0	0	0
<i>R. marina 5*</i>	3.6 (6)	469.3	248.3	53	2	4	0	0	1	23.7%
<i>R. marina 8</i>	4.0 (8)	162.0	46.90	29	2	3	0	0	0	3.4%
<i>R. marina 10</i>	4.0 (8)	103.2	40.70	39	2	3	0	0	0	0
<i>R. marina 11</i>	4.0 (8)	206.8	135.1	65	2	3	0	0.8	0	11.2%
<i>R. marina 12</i>	4.0 (8)	56.50	32.35	57	2	4	0	0	0	0.9%
<i>R. marina 14</i>	3.0 (6)	208.8	48.60	22	1	1	0	0	0	0
<i>Leptodactylus rhodomystax</i>	3.0 (6)	59.70	38.70	65	2	2	0	0	0	0
<i>L. rhodomystax 2</i>	3.0 (6)	14.00	5.30	38	2	3	0	0	0	14.3%
<i>Oxyrhopus melanogenys</i>	15.0 (30)	62.42	26.91	43	4	4	0	0.3	0	0
<i>O. petolarius</i>	4.0 (8)	67.80	12.90	19	2	4	0	7	0	0
<i>O. petolarius 2</i>	1.3 (3)	57.00	16.40	29	2	3	0	5	0	0
<i>Xenodon severus</i>	5.0 (10)	209.0	42.50	20	1	3	0	0.3	0	39.2%
<i>L. achesis muta</i>	11.0 (22)	50.70	26.35	52	1	1	0	0	0	0
<i>Epicrates cenchrria</i>	1.2 (3)	5.50	5.00	91	2	3	0	0	0	0
<i>Platemys platycephala</i>	3.0 (6)	170.8	51.40	30	4	5	0	0.2	4	47.2%
Average	4.6 (9.3)	159.2	64.80	43.1	2.1	3.1	0.1	0.8	-	-

including one individual (*X. severus*) that moved nearly 40% of its total EDM in water. We observed a wide variation in movement distances between species (Table 2), with some individuals moving almost the full 500 m within 3.5–4.5 d and others moving < 65 m over 11–15 d. On average, we collected detailed ecological data over an average of 4.6 d (range 1.25–15 d) and recorded movement distances of an average of 159 m (range 5.5–469.3 m).

Comparative assessment of method.—The cost of one bobbin is just under £0.20 (about \$0.28 USD; minimum order of 200 purchased twice for this study), resulting in £80 (about \$113 USD) of costs. Further equipment cost approximately £90 (about \$127 USD), making a total cost of £170 (about \$240 USD). If we had used external radio-transmitters, then 29 transmitters would have been needed at £92 (about \$130 USD)/transmitter (weight range: 2.0–3.8 g, longevity of up to 6 mo; Holohil Systems Ltd., Ontario, Canada). With the extra costs of a portable receiver (£466: about \$660 USD; TR-4,

Telonic Inc., Mesa, Arizona, USA) with antenna (approximately £100: about \$142 USD), the total would be approximately £3,234 (about \$4,579 USD). Furthermore we calculated, based on the above costs, that the external transmitters cost were approximately £0.25 (about \$0.35 USD)/relocation (6 mo = 364 relocations) and thread bobbins cost were approximately £0.021 (about \$0.03 USD)/relocation, based on the average number of relocations (9.3) in this study.

DISCUSSION

Overall, our results demonstrate that the thread bobbin method is suitable for use as a rapid ecological survey method in tropical rainforests with successful tracking data that can be collected for a range of different types of herpetofauna. The results also highlighted six species for which the method is currently not suitable, as well as issues encountered with the method during the tracking of individuals from three species we did successfully track during the study. Our results also show that this

tracking technique provides much more accurate information on movement distances than would be possible using alternative methods that rely only on SLD relocation data, as well as greater detail on habitat use. Furthermore, we show that this method is inexpensive and simple to use compared with more conventional techniques such as radio tracking.

Test of bobbin method.—We show the method to be successful for medium to large terrestrial species that may occasionally use aquatic and semi-arboreal habitats. The range of different snake species tracked (highly muscular to long and slender, having smooth to keeled scales) shows the methods versatility within this key group in which ecological information is particularly sparse. The numbers of successfully tracked anurans shows the success for two species of large-bodied amphibians (*Rhinella marina* in particular) and, furthermore, the method was successful with a semi-aquatic testudine. Smaller, lighter bobbins would facilitate the attachment of smaller species, and Danfield Ltd manufacturers informed us that it was possible to produce 1 g (65 m) and 1.5 g (100 m) bobbins (£65; about \$92USD/kg, minimum of 3 kg).

The results also highlighted limitations associated with the method within this habitat. There is the possibility of error in SLD calculated using GPS coordinates on Google Earth due to both GPS inaccuracy and Google Earth software errors. However, we used this approach only five times (of 76 relocations were the animal moved) and when analyses were re-run to include the average GPS error (± 16 m), the results were still significant ($P < 0.001$). The duct tape lost effectiveness in persistently wet conditions; therefore, other adhesives, such as superglues may better facilitate adhesion (Madrid-Sotelo and Garcia-Aguayo 2008). There is the possibility that the presence of the device may be detrimental to those individuals that we did not recaptured. However, it is likely that they will escape due to duct tape losing its effectiveness with no long-term impact upon the animal (Richards et al. 1994). The presence of the device may increase predation risk (Blomquist and Hunter 2007), which we recorded once in this study. However, considering that this was a unique occurrence, this could be due to chance predation and not necessarily attributable to the tracking attachment. The presence of the researcher during relocation may influence animal behavior (Ward et al. 2013) and thus could bias results. However, all tracking methods, with the exception of automated radio telemetry, require regular relocation and therefore this is not exclusively a disadvantage of the thread bobbin method. We took steps to reduce disturbance by keeping at least 2 m from the animal during relocations. The length of the thread limits the distance over which someone can track an animal. The replacement of the

thread bobbin as the thread neared the end (as demonstrated here for one individual) could extend the length of tracking. However, this approach would also increase the potential impact on the animal due to increased handling.

Effectiveness of thread bobbin method at gathering ecological data.—Measuring the distance along the thread (EDM) was found to be a truer representation of the activity of an animal than measuring simple straight-line distances (SLD). This was especially true for active individuals that occasionally use small areas, perhaps looking for an appropriate retreat site or leaving a retreat site to feed and then return. We observed this pattern multiple times within the study and it increased the EDM but made little or no difference to the SLD. Recording details along the thread trail allowed for us to gather data on how animals used their habitat when active. As almost all tracked individuals in this study were resting when relocated. This is important information that would otherwise be left unknown but may be of crucial importance when considering specific management and conservation plans of such species. Useful ecological information recorded using thread bobbins in this study included detailed information on arboreal movements, substrate use, and aquatic movements, with our results showing habitat preferences of specific species (e.g., arboreal and aquatic movements in *O. petolarius* and *X. severus*, respectively) that were clearly recorded along the thread trail but would likely be undetected with traditional tracking methods.

Comparative assessment of methods.—The use of thread bobbins is a cost effective tracking method that can gather detailed ecological data over a short term study (Table 3). It is ideal for rapidly surveying a tropical rainforest habitat. Alternative tracking methods, such as radio tracking are expensive in comparison and do not provide the same depth of ecological information over the short term (Key and Woods 1996). Assumptions on the path of movement may be made; however, our results have shown that ecological conclusions drawn from looking at relocation-only data could be inaccurate. The use of semi-arboreal and aquatic habitats by *O. petolarius* and *X. severus* are examples from this study of the ecological information that can be gain from using the thread bobbin method over others. Such ecological information is necessary to know to identify key life-history traits before considering future conservation plans to maximum success (Griffith et al. 1989). Fluorescent powders may provide similar depth of ecological information, but they are limited in their longevity with maximum recorded distances of only 17 m and 60 m in a tropical rainforest habitat for an amphibian and a mammal species, respectively (compared to a maximum of 469.3 m in this

TABLE 3. Comparison of ecological survey methods used for herpetofauna in tropical rainforests. The information was compiled primarily based on Heyer et al. (1994), Beausoleil et al. (2004), McDiarmid et al. (2012), and studies using the method with herpetofauna and/or in tropical rainforest, as well as observations and conclusions from data collected within this study. The categories low, medium, and high are qualitative scores explained specifically after each category placement and in the methods separately for each variable.

	Radio-transmitters		Automated radio telemetry	Radioactive isotopes	Fluorescent powders	Thread bobbins
	Internal	External				
Equipment costs	Medium-High < £1,000 (about \$1,416 USD) ^{1,2}		High > £1,000 (about \$1,416 USD) ^{3,4}	Medium-High < £1,000 (about \$1,416 USD) ⁵	Low < £100 (about \$142 USD) ^{6,7}	Low < £100 (about \$142 USD) ^{8,9}
Longevity	High: months-years ¹		High: months-years ³	High: months-years ¹⁰	Low: days ^{7,11}	Low: days to weeks ^{9,12}
Detail of data on microhabitat use	Medium - exact relocation site repeatedly recorded ^{13,9}		Medium - relocation to within 30-142 m ^{3,4}	Medium - exact relocation site repeatedly recorded ⁵	High - exact movements recorded ^{7,14}	High - exact movements recorded ^{9,12}
Detail of data on activity	Low-Medium - exact relocation site repeatedly recorded ^{13,9}		High - almost real-time activity data ^{3,4}	Low-Medium - exact relocation site repeatedly recorded ⁵	Medium - activities and behaviors recorded at and potentially between relocations ^{7,15}	Medium - activities and behaviors recorded at and potentially between relocations ⁹
Suitability over large distances	Medium-High - movements over km, through increased effort, dense vegetation decreases signal ^{3,16}		High - movements over km automatically recorded ^{3,4}	Low - difficult to locate over wide area ⁵	Low - less than 100m ^{11,15}	Low-Medium - movements up to 500m
Potential impact	High - surgery/force-feeding and relocation ^{4,13,17}	Medium - device attachment and carrying and relocation ^{4,5,17}	Low-Medium - device attachment and carrying ^{3,4}	High - implantation/injection, radioactive material and relocation ¹⁸	Low - relocation ^{7,19}	Medium - device attachment and carrying and relocation ²⁰
Size minimum of animal (g)	4g ³		4g ³	Very small ⁵	No minimum ⁷	60g

¹Holohil Systems Ltd, Ontario, Canada; ²Telonic Inc., Arizona, US; ³Kays et al. 2011; ⁴Ward et al. 2013; ⁵Ashton 1994; ⁶DayGlo Color Corporation, Cleveland, Ohio; ⁷Furman et al. 2011; ⁸Danfield Ltd., Leigh, UK; ⁹Tozetti and Martins 2007; ¹⁰Ashton 1975; ¹¹Lindquist et al. 2007; ¹²Stickel 1950; ¹³Richards et al. 1994; ¹⁴Stark and Fox 2000; ¹⁵Nicolas and Colyn 2007; ¹⁶Cresswell 2005; ¹⁷Plummer and Ferner 2012; ¹⁸Mellor et al. 2004; ¹⁹Dodd 1992; ²⁰Heyer 1994.

study). Furthermore, habitat complexity, humidity, and frequent rain within a rainforest limit the suitability of fluorescent powders in this environment (Nicolas and Colyn 2007). No expertise is necessary with the thread bobbin method, unlike radioactive isotopes or internal radio-transmitters, and the method would not be restricted by any issues encountered when using electronics in humid and dense tropical rainforests as it requires basic equipment. In comparison, the ease of the thread bobbin method along with the ecological information it provided and its very low costs, means that this technique is a useful tool when studying the ecology of rainforest species.

Conclusions.—Tropical rainforests have a highly complex three-dimensional structure in which microhabitat use of burrows and logs, as well as arboreal and aquatic environments, are pivotal aspects in the ecology of many rainforest species. Therefore, the ability of this tracking method to provide information about the finely detailed movements of species through these features makes it a highly relevant tool when studying the ecology of tropical rainforest species. This study has displayed the suitability of the thread bobbin method for a range of species. However, there is great potential for its use on other rainforest herpetofauna species (e.g., medium to large lizards and tortoises), as well as potentially being highly applicable for a wide range of tropical rainforest taxa. Mammals and

invertebrates have successfully been tracked using this method in different environments (Key and Woods, 1996; Cunha and Vieira 2002; Steinwald et al. 2006; Schlacher and Lucrezi 2010; Meyer and Cowie 2011) and studies investigating the ecology of suitably sized rainforest taxa might also consider this method as a way to provide greater in-depth information. Given the low cost, it would be worthwhile having the necessary materials readily available to use on focal species when the opportunity arises, thus maximizing the amount of ecological data that can be collected when there is a natural scarcity of encounters within short field seasons and difficulty of sampling in the tropics.

Developing methods that allow for rapid collection of ecological data on tropical rainforest taxa will provide valuable information on species, leading to more detailed and informative assessment of populations over time and better evaluations to predict whether species are in need of management or conservation actions. Basic ecological information provides a starting point to understanding the life-history traits of a species that are necessary for management and conservation strategies. In this study, five of the eight species that we tracked have yet to be evaluated by the IUCN, demonstrating the severe lack of basic knowledge of tropical herpetofauna populations.

Acknowledgments.—Thanks to The Crees Foundation and, in particular, director Quinn Meyer for enabling us carry out data collection at the Manu Learning Centre and for providing us with a discounted research rate through the Amazon Scholarship Programme. Special thanks to field assistant Simone Bowie, Crees volunteers, and Crees' research field team (Jaime Villacampa, Grant Reekie, Alex Fowler and Nelson Coila), who helped gather field data. Thanks to all other staff at the MLC and Crees' Cusco and London offices for their logistical support. Thanks to the Ministerio de Agricultura of Peru for providing Andrew Whitworth the permit to conduct research in Peru (Permit Number: 25397; Authorisation Number: 2904-2012-AG-DGFFS-DGEFFS). Ross MacLeod was supported by a Royal Society of Edinburgh Scottish Government Research Fellowship.

LITERATURE CITED

- Ashton Jr, R.E. 1975. A study of movement, home range, and winter behavior of *Desmognathus fuscus* (Rafinesque). *Journal of Herpetology* 9:85–91.
- Ashton Jr, R.E. 1994. Tracking with radioactive tags. Pp. 158–163 *In* Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.A.C. Hayek, and M.S. Foster (Eds.). Smithsonian Institution Press, Washington, D.C., USA.
- Beaupre, S.J., and L.E. Douglas. 2009. Snakes as indicators and monitors of ecosystem properties. Pp. 244–261 *In* Snakes: Ecology and Conservation. Mullen, S.J. and R.A. Seigel. (Eds.). Cornell University Press, New York, New York, USA.
- Beausoleil, N.J., D.J. Mellor, and K.J. Stafford. 2004. Methods for marking New Zealand wildlife: amphibians, reptiles and marine mammals. New Zealand Department of Conservation, Wellington, New Zealand.
- Beirne, C., O. Burdekin, and A. Whitworth. 2013. Herpetofaunal responses to anthropogenic habitat change within a small forest reserve in eastern Ecuador. *The Herpetological Journal* 23:209–219.
- Blankenship, E.L., T.W. Bryan, and S.P. Jacobsen. 1990. A method for tracking tortoises using fluorescent powder. *Herpetological Review* 21:88–89.
- Blomquist, S.M., and M.L. Hunter. 2007. Externally attached radio-transmitters have limited effects on the antipredator behaviour and vagility of *Rana pipiens* and *Rana sylvatica*. *Journal of Herpetology* 41:430–438.
- Böhm, M., B. Collen, J.E.M. Baillie, P. Bowles, J. Chanson, N. Cox, G. Hammerson, M. Hoffmann, S.R. Livingstone, M. Ram, et al. 2013. The conservation status of the world's reptiles. *Biological Conservation* 157:372–385.
- Collins, J.P., and A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. *Diversity and Distributions* 9:89–98.
- Cresswell, B. 2005. Practical Radio-tracking. Biotrack Ltd., Wareham, Dorset, UK.
- Cunha, A.A., and M.V. Vieira. 2002. Support diameter, incline, and vertical movements of four didelphid marsupials in the Atlantic forest of Brazil. *Journal of Zoology* 258:419–426.
- Díaz-Paniagua, C., C. Keller, and A.C. Andrew. 1995. Annual variation of activity and daily distances moved in adult Spur-thighed Tortoises, *Testudo graeca*, in Southwestern Spain. *Herpetologica* 51: 225–233.
- Dole, J.W. 1965. Summer movements of adult Leopard Frogs, *Rana pipiens* (Schreber), in northern Michigan. *Ecology* 46:236–255.
- Dodd, C.K., Jr. 1992. Fluorescent powder is only partially successful in tracking movements of the Six-lined Racerunner (*Cnemidophorus sexlineatus*). *Florida Field Naturalist* 20:8–14.
- Duellman, W.E. 1999. Patterns of Distribution of Amphibians: A Global Perspective. The Johns Hopkins University Press, Baltimore, Maryland, USA.
- Duellman, W.E. 2005. Cusco Amazonico: The Lives of Amphibians and Reptiles in an Amazonian Rainforest. Comstock Publishing Associates, Cornell University Press, Ithaca, New York, USA.

- Eggert, C. 2002. Use of fluorescent pigments and implantable transmitters to track a fossorial toad (*Pelobates fuscus*). *Herpetological Journal* 12:69–74.
- Foster, M.S., R.W. McDiarmid, and N. Chernoff. 2012. Studying reptile diversity. Pp. 3–5 *In* *Reptile Biodiversity: Standard Methods for Inventory and Monitoring*. McDiarmid, R.W., M.S. Foster, C. Guyer, J.W. Gibbons, and N. Chernoff (Eds.). University of California Press, Berkeley, California, USA.
- Furman, B.L.S., B.R. Scheffers, and C.A. Paszkowski. 2011. The use of fluorescent powdered pigments as a tracking technique for snakes. *Herpetological Conservation and Biology* 6:473–478.
- Gibbons, J.W., D.E. Scott, T.J. Ryan, K.A. Buhlmann, T.D. Tuberville, B.S. Metts, J.L. Greene, T. Mills, Y. Leiden, S. Poppy, and C.T. Winne. 2000. The global decline of reptiles, déjà vu amphibians. *Bioscience* 50:653–666.
- Griffith, B., J.M. Scott, J.W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. *Science* 245:477–480.
- Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.A.C. Hayek, and M.S. Foster (Eds.). 1994. *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, D.C., USA.
- Heyer, W.R. 1994. Thread bobbins. Pp. 153–155 *In* *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.A.C. Hayek, and M.S. Foster (Eds.). Smithsonian Institution Press, Washington, D.C., USA.
- Hillman, S., P. Withers, R. Drewes, and S. Hillyard. 2009. *Ecological and Environmental Physiology of Amphibians*. Oxford University Press, Inc., New York, New York, USA.
- Kay, W.R. 2004. Movements and home ranges of radio-tracked *Crocodylus porosus* in the Cambridge Gulf region of Western Australia. *Wildlife Research* 31:495–508.
- Kays, R., S. Tilak, M. Crofoot, T. Fountain, D. Obando, A. Ortega, F. Kueemeth, J.M. Mandel, G. Swenson, T. Lambert, et al. 2011. Tracking animal location and activity with an automated radio telemetry system in a tropical rainforest. *The Computer Journal* 54:1931–1948.
- Key, G.E., and R.D. Woods 1996. Spool-and-line studies on the behavioural ecology of rats (*Rattus* spp.) in the Galapagos Islands. *Canadian Journal of Zoology* 74:733–737.
- Lindquist, E.D., S.A. Sapochnik, E.J.G. Rodriguez, P.B. Johantgen, and J.M. Criswell. 2007. Nocturnal position in the Panamanian Golden Frog, *Atelopus zeteki* (Anura, Bufonidae), with notes on fluorescent pigment tracking. *Phyllomedusa* 6:37–44.
- Lips K.R. 1998. Decline of a tropical montane amphibian fauna. *Conservation Biology* 12:703–707.
- Madrid-Sotelo, C.A., and A. Garcia-Aguayo. 2008. A simple method for externally attaching radio-transmitters to snakes. *North-Western Journal of Zoology* 4:335–338.
- McDiarmid, R.W., M.S. Foster, C. Guyer, J.W. Gibbons, and N. Chernoff (Eds.). 2012. *Reptile Biodiversity: Standard Methods for Inventory and Monitoring*. University of California Press, Berkeley, California, USA.
- Mellor, D.J., N.J. Beausoleil, and K.J. Stafford. 2004. Marking amphibians, reptiles and marine mammals: animal welfare, practicalities and public perceptions in New Zealand. New Zealand Department of Conservation, Wellington, New Zealand.
- Meyer, W.M., and R.H. Cowie. 2011. Distribution, movement, and microhabitat use of the introduced predatory snail *Euglandina rosea* in Hawaii: implications for management. *Invertebrate Biology* 130:325–333.
- Munger, J.C. 1984. Home ranges of horned lizards (*Phrynosoma*): circumscribed and exclusive? *Oecologia* 62:351–360.
- Nicolas, V., and M. Colyn. 2007. Efficiency of fluorescent powder tracking for studying use of space by small mammals in an African rainforest. *African Journal of Ecology* 45:577–580.
- Plummer, M.V., and J.W. Ferner. 2012. Marking reptiles. Pp. 143–150 *In* *Reptile Biodiversity: Standard Methods for Inventory and Monitoring*. McDiarmid, R.W., M.S. Foster, C. Guyer, J.W. Gibbons, and N. Chernoff (Eds.). University of California Press, Berkeley, California, USA.
- R Core Team. (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at <http://www.R-project.org>.
- Richards, S.J., U. Sinsch, and R.A. Alford. 1994. Radio tracking. Pp. 155–158 *In* *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.A.C. Hayek, and M.S. Foster (Eds.). Smithsonian Institution Press, Washington, D.C., USA.
- Rittenhouse, T.A.G., T.T. Altnether, and R.D. Semlitsch. 2006. Fluorescent powder pigments as a harmless tracking method for Ambystomatids and Ranids. *Herpetological Review* 37:188–191.
- Rowley, J.J., and R.A. Alford 2007. Movement patterns and habitat use of rainforest stream frogs in northern Queensland, Australia: implications for extinction vulnerability. *Wildlife Research* 34:371–378.
- Schlacher, T.A., and S. Lucrezi. 2010. Compression of home ranges in Ghost Crabs on sandy beaches

Herpetological Conservation and Biology

- impacted by vehicle traffic. *Marine Biology* 157:2467–2474.
- Stark, R.C., and S.F. Fox. 2000. Use of fluorescent powder to track horned lizards. *Herpetological Review* 31:230–231.
- Stark, R.C., S.F. Fox, and D.M. Leslie, Jr. 2005. Male Texas Horned Lizards increase daily movements and area covered in spring: a mate searching strategy? *Journal of Herpetology* 39:169–173.
- Steinwald, M.C., B.J. Swanson, and P.M. Waser. 2006. Effects of spool-and-line tracking on small desert mammals. *Southwestern Naturalist* 51:71–78.
- Stickel, L.F. 1950. Populations and home range relationships of the box turtle, *Terrapene c. carolina* (Linnaeus). *Ecological Monographs* 20:351–378.
- Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman, and R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783–1786.
- Thompson, G. 1993. Daily movement patterns and habitat preferences of *Varanus caudolineatus* (Reptilia: Varanidae). *Wildlife Research* 20:227–231.
- Tozetti, A.M., and M. Martins. 2007. A technique for external radio-transmitter attachment and the use of thread-bobbins for studying snake movements. *South American Journal of Herpetology* 2:184–190.
- Tozetti, A.M., and L.F. Toledo. 2005. Short-term movement and retreat sites of *Leptodactylus labyrinthicus* (Anura: Leptodactylidae) during the breeding season: a spool-and-line tracking study. *Journal of Herpetology* 39:640–644.
- Ward, M.P., J.H. Sperry, and P.J. Weatherhead. 2013. Evaluation of automated radio telemetry for quantifying movements and home ranges of snakes. *Journal of Herpetology* 47:337–345.
- Wasko, D.K., and M. Sasa. 2009. Activity patterns of a neotropical ambush predator: spatial ecology of the Fer-de-lance (*Bothrops asper*, Serpentes: Viperidae) in Costa Rica. *Biotropica* 41:241–249.
- Whitworth, A., Downie, R. von May, R., Villacampa, J. and MacLeod, R. 2016. How much potential biodiversity and conservation value can a regenerating rainforest provide? A ‘best-case scenario’ approach from the Peruvian Amazon. *Tropical Conservation Science* 9:211–232.



EMILY WADDELL graduated from the University of Glasgow, UK, with a B.S. in Zoology in 2010 and again in 2012 with a Master’s of Research in Ecology and Environmental Biology. She currently works for U.K. based wildlife charity Froglife and is due to start a Ph.D. at Centre for Ecology and Hydrology and University of York, U.K. in October, focusing on plant invasion of tropical forest fragments. (Photographed by Andrew Whitworth).



ROSS MACLEOD is Research Fellow at the Institute of Biodiversity, Animal Health, and Comparative Medicine at the University of Glasgow, UK, where he graduated in Zoology in 1999, before moving to the University of Oxford to complete a Ph.D. His current research focuses on using ecological theory and individual behavioral responses to understand and predict future responses to environmental change and on developing new biodiversity survey methods to promote the collection of reliable scientific data that can be used for conservation. (Photographed by Mel MacLeod).



ANDREW WHITWORTH received his B.S. in Biology from the University of Leeds, UK, his M.S. from Manchester Metropolitan University, and recently completed his Ph.D. at The University of Glasgow. Andrew’s major interest lies within areas of tropical forest that were once subjected to human habitat alteration and understanding the patterns of biodiversity found within these areas as they regenerate. (Photographed by Marcus Brent-Smith).