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# Correspondence

## Use of the spool-and-line technique for studying microhabitat selection and daily movement of snakes in the Atlantic Forest of Brazil

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Movement can be defined as a change in the spatial position of an individual through time (Nathan et al. 2008). This change depends on the obstacles imposed by the environment, such as soil conditions, relationships with other individuals, resource distribution, and the individual's ability to surmount these obstacles (Nathan et al. 2008, Preisler et al. 2004). In addition, the success or failure of individuals' movements determines population and community structure and dynamics (Root & Kareiva 1984, Spiegel et al. 2017, Eskew & Todd 2017). Studies on movement ecology have been increasing in recent years and they aim to understand the causes, mechanisms, and spatiotemporal patterns of movement, and their role in various ecological and evolutionary processes (Getz & Saltz 2008, Nathan et al. 2008). Despite these studies having been conducted for diverse vertebrate classes (Avgar et al. 2013), they are still scarce for snakes.

Some techniques have been developed to provide detailed information on animal movement patterns and microhabitat preferences, and the most successful ones used to track species of herpetofauna are radio-telemetry (Shine et al. 2003, Tozetti et al. 2009), fluorescent powder technique (Furman et al. 2011, Plummer & Ferner 2012), and employing spool-and-line devices (Tozetti & Toledo 2005, Tozetti & Martins 2007). Radio-tracking can be considered the most common and promising method for studies of the spatial ecology of snakes (Ward et al. 2013) owing to the possibility of locating individuals that were captured earlier. However, this method entails some negative aspects, such as the high cost of the telemetry equipment and the difficulty in attaching the transmitters to some snakes, in particular small ones (Tozetti & Martins 2007), and because this method provides only location points, but no trajectories and distances travelled. The fluorescent powder technique involves covering the body of an animal with UV powder and following the UV traces that are left with a blacklight spotlight (Plummer & Ferner 2012). Although this method has been successfully used to study a range of herpetofauna (Blankenship et al. 1990, Stark & Fox 2000, Eggert 2002, Rittenhouse et al. 2006, Furman et al. 2011) its maximum tracking distance is limited, and it does not work well in forests due to the trail being broken when a tracked arboreal snake moves from one branch or tree to another (Waddell et al. 2016). For its part, the spool-and-line device or thread-bobbins method is low-cost and can provide a highly detailed description of snake movement, such as the exact route followed by the animal, as well as the true distance travelled between encounter points (Vieira & Loretto 2005, Tozetti & Martins 2007).

The spool-and-line technique was originally proposed by Breeder (1927) for chelonians, refined by Miles (1976), and has since been used with different animals such as mammals (Vieira & Cunha 2002, Delciellos et al. 2018), anurans (Tozetti & Toledo 2005), turtles (Famelli et al. 2016), lizards (Sanches & Grings 2018, Law et al. 2016), snakes (Tozetti et al. 2009, Waddell et al. 2016), and even snails (Murphy 2002). However, the exact manner in which the thread-bobbins are attached is different for each group, and few studies detail the procedures to apply this technique properly (Vieira & Loretto 2005, Tozetti & Martins 2007). We used spool-and-line devices to monitor the movements of 48 snakes, representative of 20 species, in the northeastern Atlantic Forest of Brazil. We here describe the application of this method and discuss its efficiency for snakes of different species, sizes, and habits.

The spools we used are polyester cocoons (produced in Brazil by Hiltex Indústria e Comércio de Fios Ltda.), 1.3 cm in length, and weighing approximately 3–5 g for 250 m of line. The line pays out smoothly and with minimal resistance from the inside of the cocoon (Fig. 1). The cocoon is attached to the snake by means of a wrap of duct tape (Silver Tape 3M Scotch TM 45 × 25 mm.) after it has been enveloped in plastic foil to avoid the line sticking to the tape (Fig. 2). The duct tape wrap is fixed 5 cm above the animal's cloaca with a complete loop; applying more than one loop is not advisable because it would increase the weight of the wrap. The duct tape will not lose its stickiness even in water, and the wrap will detach from the snake during its next moult.

Figure 1. A polyester cocoon bobbin as used in this work.

It is necessary to immobilize the snakes to attach the thread-bobbins, and the most efficient approach here is to use transparent PVC tubes of adequate diameters. Two people are needed for this step: one person is to keep the anterior portion of the snake inside the tube and hold the snake still where the wrap is to be affixed. The second person is to affix the cocoon carefully in the first attempt, because once affixed, the tape cannot be detached easily anymore and attempting to do so may cause injuries to the animal's skin. After the wrap is fixed, the opening in the upper extremity of the cocoon must be closed with a piece of tape to prevent sand and other debris from accumulating in the wrap. After the thread-bobbin has been attached thus, the snake can be released in the forest with the loose end of the line tied to the trunk of a shrub, a branch, or a firmly anchored object on the ground.

We observed that most snakes first showed an erratic behaviour when released, such as rolling around themselves a couple of times. However, all of them returned to normal movements within less than a minute and then moved away. From then on, the spool pays out line, leaving a trail of where the snake has crawled and describing the trajectory of its movements (Fig. 3).

The spool-and-line method provides individual ecological data, such as movement and locomotion patterns,

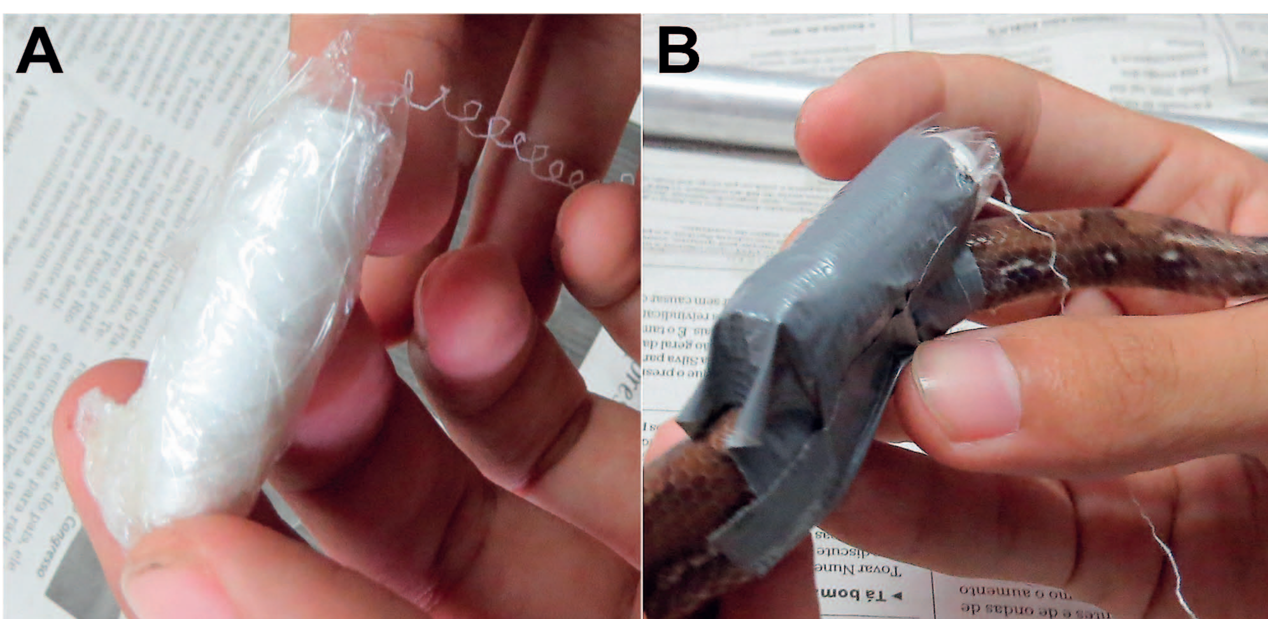


Figure 2. The cocoon covered in PVC foil (A) and the wrap attached to *Epicrates assisii* (B).

microhabitat use, and behaviour of the individual animals monitored (Vieira & Loretto 2005). However, to ensure the informative reliability of these data, it is necessary to take some precautions to minimize possible interferences. One of these concerns the weight of the snake. Waddell et al. (2016) recommend that the total weight of the wrap not exceed 10% of the animal's weight. The cocoon is 3.5 g and adding the tape and plastic foil (approximately 1.2 g), the weight of the wrap reaches close to 5 g. If such a spool is attached to animals weighing less than 50 g, it could compromise the animal. This 10% security margin was assured for all 48 animals tracked successfully in this study.

Nevertheless, a spool-and-line device can be used in smaller and lighter snakes. In their cases, we recommend that both tape and spool be reduced in volume until their combined weight is compatible with the weight of the animal. The spool has two extremities, one of which unwinds from the inside of the cocoon and the other from the outside; the best way to reduce the weight of the spool is by pulling off thread from the outside extremity until the desired weight is reached. This approach lends itself to tracking neonates, juveniles, and small-sized species, but we found that it will reach the limit of its feasibility when the weight of the wrap is brought down to 0.8 g, because the spool will have only approximately 10 m of line remaining. While this may still be enough to track small-sized snakes and juveniles for a few days, we found it to be of limited use in the case of racer snakes (see below).

For large snakes or those with intense daily movements, such as racer snakes, we applied a second spool whose inner end of thread was knotted to the outer one of the first spool in this manner doubled the length of the line. The use of two spools is of course limited by the increased weight and size of the wrap. We tested it successfully in large specimens of the genera *Philodryas*, *Bothrops*, *Boa,* and *Spilotes* by increasing monitoring times for them. We used only two connected spools, but, depending on the size of the animal, more than two spools may be linked, as has been demonstrated by Law et al. (2016) in a study of the large *Varanus bitatawa* Welton et al., 2010.

Besides the weight of the wrap, its size can impair some slender species whose movements may be affected by having to wear a "backpack" or "splint". We also investigated the effects of attaching the wrap to other parts of the snake's dorsum, but found that attaching it approximately 5 cm forward above the animal's cloaca to the snake's dorsum had the least interference on its movement. This positioning was the same as that used by Tozetti & Martins (2007) in a study of the Brazilian rattlesnake, but it was

Figure 3. *Boa constrictor* in the field with the spool-and-line attached.

quite different from that used by Waddel et al. (2016) who attached the spool laterally in the posterior region of the snake's body.

We found this method to be of limited use in the cases of some slender arboreal snakes, such as *Chironius* and *Thamnodynastes*. These animals frequently curl the body in coils around branches and vines to move, and the "splint" can restrict the formation of one or more coils, making movement difficult or impossible. We tested the method for these arboreal species by reducing the spool to the recommended weight and reducing the wrapping tape to a size not exceeding 7 to 10% of the snout–vent length of the snake. This reduction worked successfully in *Chironius*, but failed to improve the free movement in *Thamnodynastes*. Another difficulty in tracking arboreal snakes using the spool-and-line technique is not related to the attachment of the spool, but to their habits and behaviour. Large arboreal snakes, such as *Spilotes* and *Boa*, can climb the highest trees of the forest and remain hidden from sight, for which reason it may sometimes be difficult to follow the line, even with the aid of binoculars.

Another precaution to be observed in studies with spool-and-line techniques is the the tracker possibly interfering in the movement or disturbing the rest of the animal. In various situations the tracker may not be able to see the snake owing to its cryptic habits. This can lead the researcher to the snake's hiding place and, for example, cause it to leave its resting place. The researcher may furthermore stimulate non-standard locomotion or forced movement in response to his approach. To ensure the reliability of the data, we recommend that the tracker avoid making too much contact with the snake. In our study, many snakes presented sedentary behaviour, in some instances remaining hidden for more than 15 days.

The spool-and-line technique is a cheap and easy-to-use method for tracking snakes. Aside from the experienced field herpetologist obtaining ecological data using this method, other people may also find the line, follow it, and find the snake at its end. This could be very harmful for the snakes in many places. Therefore, an important factor to be considered is human activity and frequency in the study area, and we strongly recommend avoiding this technique in more densely urbanized areas with free public access. Not only people, but also pets such as cats and dogs can follow or destroy the line and ruin the research.

From October of 2017 to October of 2018 we performed active searches, once per week, with a total of 576 manhours of search, in the Guaribas Biological Reserve – a fragment of Atlantic Forest with an area of approximately 327 hectares, adjacent to the urban area in Rio Tinto county, Paraiba State, Brazil (Mesquita et al. 2018). We captured 72 snakes during this period, of which 28 (39%) were considered unsuitable to testing the spool-and-line method and 44 (61%) suitable test candidates. The 28 snakes deemed unsuitable did not conform to the 10% weight rule, even after reduction of spool size. They were two individuals each of *Apostolepis cearensis* Gomes, 1915 and *Taeniophallus affinis* (Günther, 1858), three small juveniles of *Bothrops leucurus* Wagler, 1824, three juveniles of *Erythrolamprus almadensis* (Wagler, 1824), one juvenile of *Erythrolamprus poecilogyrus* (Wied-Neuwied, 1825), two juveniles of *Erythrolamprus viridis* (Günther, 1862), five juveniles of *Dipsas mikanii* (Schlegel, 1837), one individual of *Tantilla melanocephala* (Linnaeus, 1758), and nine individuals of *Amerotyphlops paucisquamus* (Dixon, 1979). The other 44 snakes conformed to the weight rule; however, seven individuals of *Helicops angulatus* (Linnaeus, 1758) and two of *Micrurus ibiboboca* (Merrem, 1820) were excluded because these species were considered altogether unsuited for this method.

Spool-and-line tracking was successfully tested in 35 snakes (43%) of 14 species: seven individuals of *Boa constrictor* Linnaeus, 1758; five of *Bothrops leucurus*; six of *Chironius flavolineatus* Jan, 1863; three of *Epicrates assisi* Machado, 1945; one each of *Erythrolamprus poecilogyrus*, *Erythrolamprus taeniogaster* (Jan, 1863), *Oxybelis aeneus* (Wagler, 1824), *Oxyrhopus petolarius* (Linnaeus, 1758), *Oxyrhopus trigeminus* Duméril, Bibron & Duméril, 1854, and *Spilotes sulphureus* (Wagler, 1824); and two each of *Philodryas nattereri* Steindachner, 1870, *Philodryas olfersii* (Lichtenstein, 1823), *Philodryas patagoniensis* (Girard, 1858), and *Pseudoboa nigra* (Duméril, Bibron & Duméril, 1854). The monitoring time of these species ranged from 3 to 40 days (mean 15 days) (Table 1). We observed the natural movements of these snakes after their release. We did not have any difficulties in tracking all the terrestrial snakes, such as *Bothrops*, *Erythrolamprus,* and *Pseudoboa*. Others, such as some individuals of *Boa* and *Spilotes*, climbed very high in trees, making it sometimes difficult to follow their track lines. But even then, we managed to track these snakes by using binoculars or by also climbing the trees. Racer snakes used up their line reserves faster than other snakes; sometimes even the neonates of some racer species, such as *Philodryas*, move long distances in a short time. We tracked one juvenile of *P. nattereri* (snout–vent length 431 mm, mass 25 g), two juveniles of *P. olfersii* (410 mm, 19.5 g, and 476 mm, 17 g), and one juvenile of *P. patagoniensis* (58.5 mm, 68 g) that relocated by more than 15 metres in a single day. We could not fully track a small juvenile of *P. olfersii* (233 mm, 9 g) and a juvenile of *Oxyrhopus trigeminus* (270 mm, 10 g), because the lines of their reduced spools (approximately 12 m) had been used up in only a couple of hours.

The method failed in the case of 14 snakes (19%) of five species. We attached the thread-bobbins to four adult individuals of the water snake *Helicops angulatus*, but all of them swam directly to mud sections of the river, dug holes, and rubbed their bodies against the substrate. This behaviour caused the wrap and the animal becoming stuck, forcing us to remove their wraps and abandoning tracking them. We also attached thread-bobbins to three coral-snakes (two *Micrurus ibiboboca* and one *M. lemniscatus* (Linnaeus, 1758)), but similar to the water snake, the technique failed owing to these species constantly digging holes and moving about underground; in this situation the wrap became stuck in the entrance of the hole, pinning the

Table 1. Monitoring times with spool-and-line devices for Brazilian Atlantic Forest snakes. The ranges are in parenthesis. N = number of individuals tracked; SVL = snout–vent length; R = reduced, N = normal, D = double; M-time: monitoring time. SVL, Mass and M-time are reported as means ± standard deviations.

| FAMILY<br>Species | N | SVL (mm) | Mass (g) | Spool | M-time (days) |
|---|---|---|---|---|---|
| BOIDAE | | | | | |
| *Boa constrictor* | 7 | 894.9±668.3 (147–2300) | 975.4±1238.4 (147–2300) | 5 N / 2 D | 11.3±9.4 (2–30) |
| *Epicrates assisi* | 3 | 403.3±299.7 (116–714) | 433.0±452.8 (69–940) | 1 R / 2 N | 21.3±13.3 (6–30) |
| VIPERIDAE | | | | | |
| *Bothrops leucurus* | 5 | 766.4±280.7 (497–1150) | 638.4±480.1 (62–1140) | 1 R / 2 N / 2 D | 12.6±13.0 (2–30) |
| COLUBRIDAE | | | | | |
| *Chironius flavolineatus* | 6 | 595.3±95.0 (450–728) | 44.8±20.2 (24–80) | 6 R | 2.0±1.3 (1–4) |
| *Oxybelis aeneus* | 1 | 79 | 54 | 1 R | 1 |
| *Spilotes sulphureus* | 1 | 1600 | 533 | 1 D | 3 |
| DIPSADIDAE | | | | | |
| *Erythrolamprus poecilogyrus* | 1 | 470 | 50 | 1 N | 4 |
| *Erythrolamprus taeniogaster* | 1 | 570 | 115 | 1 N | 25 |
| *Oxyrhopus petolarius* | 1 | 312 | 8 | 1 R | 1 |
| *Oxyrhopus trigeminus* | 1 | 270 | 10 | 1 R | 1 |
| *Philodryas nattereri* | 2 | 261.5±239.7 (92–431) | 175.0±212.1 (25–325) | 1 N / 1 D | 6.0±5.7 (2–10) |
| *Philodryas olfersii* | 2 | 595.0±261.6 (410–780) | 69.8±71.1 (20–120) | 2 N | 3.0±1.4 (2–4) |
| *Philodryas patagoniensis* | 2 | 333.0±356.4 (81–585) | 351.0±465.3 (22–680) | 1 N / 1 D | 1.5±0.7 (1–2) |
| *Pseudoboa nigra* | 2 | 655.0±233.3 (490–820) | 119.0±117.4 (36 - 202) | 1 N / 1 D | 3.0±2.8 (1–5) |

tail of the snake in place, and these animals therefore had to be freed and released. We tried to monitor two *Thamnodynastes pallidus* (Linnaeus, 1758) using the exact 10% weight rule. However, as they are slender, small tree snakes, they could no longer climb with the "backpacks" attached, and it was not possible to reduce the spool further. Finally, we attached thread-bobbins to five *Dipsas mikanii*, but they did not move after being released even when their wraps were reduced in weight. In all five instances, we waited for five days, but because the snakes remained completely immobile, we eventually removed the wraps upon which they started to move again. We were not able to identify the cause of this motionlessness.

With a success rate of 43%, the spool-and-line tracking method can be considered a useful tool for collecting relevant data about the natural history and behaviour of snakes with a great level of detail if it is applied correctly and the safety margins are respected. Such data is essential to identify key life-history traits that can be used in future conservation policies (Griffith et al. 1989, Waddell et al. 2016). Although monitoring times may be short owing to the size of the line in some small-sized species, this technique can provide field information that no other currently known monitoring method can provide (Key & Woods 1996). This method is not equally well suited for use with all species, and it is important to verify their compatibility first. Testing this method with other snake species in different environments is strongly encouraged to reveal limitations and applications.

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