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Methodological Development for Harmonic Direction Finder Tracking in Salamanders

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***Abstract.*—The ability to track the movements of an animal enables a better understanding of its behavior and ecological preferences. However, animals that are small, have smooth sensitive skin, and live in variable environments can be difficult to track. We tested several types of Harmonic Detection Finder (HDF) tags and harnesses in salamanders. We designed a harness that does not hinder the normal movements of a salamander and is difficult to remove for the bearer. We also designed a dipole that could be used both on the ground and underwater without a great decrease in detection range. These techniques are important for non-invasive behavioral studies, although the harness developed here could easily get entangled in narrow or heavily vegetated habitats.**

Key Words.—caudata; Harmonic Direction Finder; harness; tracking

Introduction

Tracking animals to understand their behavior is as old as primitive hunting practices, but the first report of tracking for scientific information dates back to 1803 when banding was used to demonstrate philopatry in Eastern Phoebes, *Sayornis phoebe* (North American Banding Council 2001). Since then, improvements in design and use allowed for fundamental breakthroughs in behavioral ecology. For example, the several hundred years-old assumption about the collective breeding of European Eels (*Anguilla anguilla*) was resolved through the use of electronic tags (Righton et al. 2016).

The Harmonic Detection Finder (HDF) is a passive tracking device adapted from avalanche rescue techniques (RECCO AB; Lidingö, Sweden). The HDF consists of an emitter producing waves that are reflected by a dipole, such as a Schottky diode. The dipole is not powered and consequently the tag can be light (about. 0.2 g). Thus, it can be used with species too small to be equipped with radio-transmitters (e.g., the Brilliant-thighed Poison Frog, *Allobates femoralis*; Pašukonis et al. 2014a; Pašukonis et al. 2014b). Furthermore, passive tracking with HDF is reliable over time because no battery is required.

HDF has been used in a variety of ecological studies on frogs, including investigations on the terrestrial habitat of the European Treefrog (*Hyla arborea*; Pellet et al. 2006), the seasonal ecology and chytrid fungus related behavior in the Common Mist Frog (*Litoria rheocola*; McNab 2015; Roznik and Alford 2015), and microhabitat use in Blacksmith Treefrogs (*Hypsiboas faber*; Oliveira et al. 2016), Suweon Treefrogs (*Dryophytes suweonensis*), and Japanese Treefrogs (*D. japonicus*; Borzée et al. 2016).

Tracking salamanders has relied primarily on subcutaneous implants, mostly because of the preference of the species for narrow hiding refuges. In one early study involving tracking salamanders, radio transmitters were surgical implanted into the Eastern Hellbender (*Cryptobranchus alleganiensi*; Stouffer et al. 1983). Subsequently, Madison (1997) implanted transmitters to study emigration in the Spotted Salamander (*Ambystoma maculatum*), and Madison and Farrand (1998) used implanted transmitters to examine habitat use in the Tiger Salamander (*A. tigrinum*). More recently, Marcec et al. (2016) examined survival rate of Chinese Giant Salamanders (*Andrias davidianus*) with implanted transmitters. Non-invasive tracking methods have not been extensively used for salamanders and those that have been used were either more time-consuming (e.g., visual tracking in the California Tiger Salamander, *A. californiense*; Loredo et al. 1996) or less reliable (e.g., fluorescent powder tracking in *A. maculatum*; Pittman and Semlitsch 2013). Our goal was to design a HDF tracking protocol for salamanders that would be effective in terrestrial and aquatic environments.

Materials and Methods

Although often used for frogs and toads, we are unaware of any harness developed specifically for salamanders. Specifically, for HDF tracking, Pellet et al. (2006) provided a detailed description of waist bands

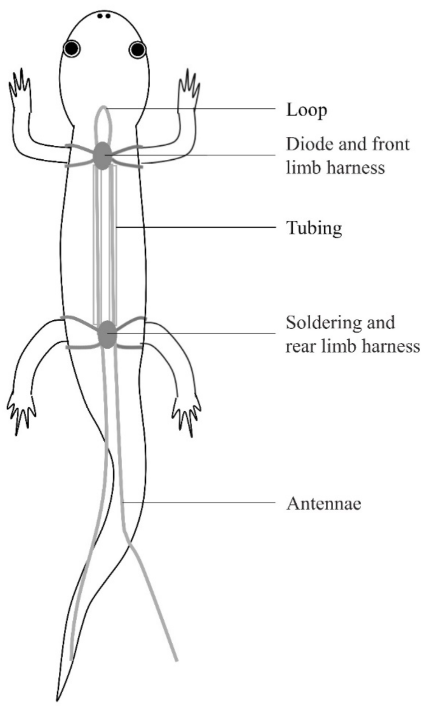

Figure 1. Diagram of harness type 5. Harnesses were made of gauze folded back on itself. The tag is made of the front loop, two wires encased in a silicone tubing wires, and the two antennae. The diode is under the stitching point for the front limbs harness and the soldering point is below the rear limb harness.

for treefrogs, but an alternative backpack harness has been used for larger anurans, such as the Wyoming Toad (*Anaxyrus baxteri*; Linhoff 2015). We integrated components of a waist bands with those of a backpack harness into a harness for salamanders.

Harness preparation.—To prevent the individual from losing the harness bearing the tag, we designed and tested several prototypes on a Tiger Salamander, which we acquired from the pet trade. To construct each harness, we folded medical-grade non-treated gauze back on itself to increase its thickness (Sanggong Yangheng #31547; Yongin, Republic of Korea). Such gauze was selected to decrease the risks of infections, and because it degrades naturally in the field within weeks (Pellet et al. 2006). For all prototypes, we placed the folded gauze around the salamander to laterally circle its body, like a belt would, and the two extremities were stitched onto each other over about 1 cm on the ventral side of the salamander. As this is designed to be conducted in the field, and optimally not lasting more than minutes, we did not anesthetize the individual. To avoid injuring the salamander, a person carefully stitched the harness around the salamander while someone else was holding it. Furthermore, we made certain that the salamander was not bloating its body as a defense mechanism and causing the harness to be loose (Fig. 1). We kept the test individual and the gauze moist through regular spraying to prevent negative health impact. We conducted all stitching and insertions explained below with the gauze folded back on itself, and these procedures rely on spaces between the warp and weft threads of the gauze. To make the insertion of the limb of the salamander into the gauze faster, we prepared the threads within a gauze piece before manipulating the animal. The size of the holes was roughly the expected diameter of the limb to insert, but we did not take special care to prevent the gauze from fraying as it was folded back and presumed sturdy.

Harness type 1.—We placed the gauze band around the body of the salamander at the front limb level, with the legs inserted in the holes of the gauze. This design was similar to that of a backpack on toads, but different in that the legs were directly inserted in the holes of the gauze.

Harness type 2.—We constructed the harness the same way as above, but the rear limbs of the salamander were inserted in the holes of the gauze. This design was similar to that of a backpack on toads but set over the rear limbs.

Harness type 3.—We constructed this harness by combining the types 1 and 2 around the front and rear limbs of the salamander. We later connected the two parts of the harness by the tag.

Harness type 4.—We prepared two half harnesses by making two connected loops of gauze for each pair of legs. We inserted each pair of legs in the matching loops and tightened by stitching the gauze ventrally two-by-two. This involved maintaining the test individual on its back for a relatively long period of time to properly stitch the gauze ventrally.

Harness type 5.—We prepared two half harnesses by making two connected loops of gauze for each pair of legs, such as for harness type 4. For each pair, we attached sewing thread to the extremity of one of the loops. We laid the two half harnesses onto the dorsal side of the salamander and then inserted all legs into the corresponding loops. We tightened the harness to the body of the salamander by tying the pre-attached threads to the opposite loop, for both front and back legs.

Harness types 1 and 2 weighed on average 0.1 g; and harnesses 3, 4 and 5 weighed on average 0.2 g, a weight within what individuals can carry without being negatively affected (Hodgkison and Hero 2001). We tested the efficiency of each harness type by assessing the

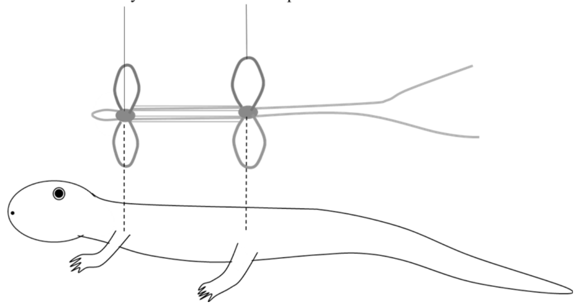

Figure 2. The Schottky diode from the antennae was positioned above the 3rd trunk vertebrae, roughly between the front limbs of the salamander. The second soldering point was roughly between the rear legs of the individuals. This step induces measuring the salamander before designing the tag, and thus tailoring it to each individual.

ability of the test individual to escape from the harness. The individual was allotted free movement for 20 min, but all escapes from the harnesses were recorded within seconds. If the harness type did not fail on the initial trial, we tested it 12 times to ensure reliability. We used a binary code (success or failure) for each harness test and used a Pearson Chi-square test (α = 0.05) in SPSS 21.0 (SPSS, Inc., Chicago, USA) to statistically test for significant differences in the usefulness of the harnesses.

Tag and antennae isolation.—For the harnesses 1 and 2, we soldered a Schottky diode (model R2; RECCO AB; Lidingö, Sweden) at a specific distance away from the bend created by folding back a tin-plated copper wire on itself (see two paragraph below for the variations in distance between bend and diode). We used a tin-plated copper wire to maintain the electric properties of the diode and the mechanical elasticity of the antennae. We cut the remaining length of wire below the diode to a length of 12 cm, which created the antennae of the tag. For these harnesses, we stitched the tag to the harness at the height of the diode.

For the harnesses 3, 4, and 5, we performed the same procedure, but instead of cutting the wire, each side of the bend, were encased into silicone tubing (1.8 mm diameter), starting from below the diode and until the space between the 3rd vertebrae and the sacrum. We soldered the two sides of the wire together as close as possible to the end of the silicone casing, while taking care not to melt the tubing. We ensured that the two wires were about 0.5 cm apart and parallel to each other, except at the soldering point where they were in contact. The soldering point was roughly at the same height as the rear legs of the individual. Down from the second soldering point, we did not encase the two sides of the wire, but we cut them at the length required for the antennae (see next paragraph for variations in length; Fig. 1). We applied a uniform coat of silicone spray (model S-830 UL94 V-0; Nabakem; Seoul, Republic of Korea) to the antennae to insulate ale the electrical components. We sealed the entrance of the silicone tubing, encasing the wires to ensure they would stay dry during the trial. To construct tags of appropriate length, we measured the salamander before creating the tag, thus tailoring it to each individual, and we positioned the Schottky diode above the 3rd trunk vertebrae, roughly between the front limbs when bound to the animal (Fig. 2). For these harnesses, we stitched the tag over the diode and the second soldering point.

Because the detection range of the tag is correlated to the length of the loop between the diode and the original bend of the wire, and because it is also correlated to the length of the antennae, we tested several combinations. We tested 12 independent replicates of the antennae with 1, 2, and 3 cm long loops and 12 cm long antennae legs (n = 36) to test for the effect of loops length. We then tested antennae with 0.4 cm long loops and 12, 15 and 25 cm long antennae legs (n = 36) to test for the effect of legs length. Both the variations in detection range for the three sizes of loops, and the variation in detection range for each of the leg lengths were tested for statistical variations using ANOVA (α = 0.05), with the loop sizes and leg lengths as dependent variables and detection distances as response variables.

Results

Harness preparation.—Only harness type 5 would securely attach the tag onto the salamander (Fig. 3). Harness type 1 was removed immediately by the salamander because the joints of the individuals were too flexible to provide support to the gauze after release. Harness types 2 and 3 were also rapidly removed by the salamander. The salamander could not remove harness type 4 when in a terrestrial environment, but this harness noticeably hindered locomotion. Furthermore, the harness was quickly removed by the salamander when in an aquatic environment. Harness type 5 was the one that we selected for repeated trials as the salamander did not remove it in either aquatic or terrestrial environments, and significantly highlighted the success of the harness type ($\chi^2 = 16.00$, df = 4, $P = 0.003$; n = 16). In addition, the movements of the salamander did not seem impaired (i.e., appropriate locomotion and swimming behaviors) over the 12 replicate tests of the harness.

Tag and antennae isolation.—We first tested the tag built with two 12 cm long legs to determine the importance of the front loop. The loops that were 1 and 2 cm long were functional, but the individual had difficulty lifting its head when using the 3 cm long loop. The average detection ranges were different for each of the loop sizes. When placed underwater, a 1 cm loop

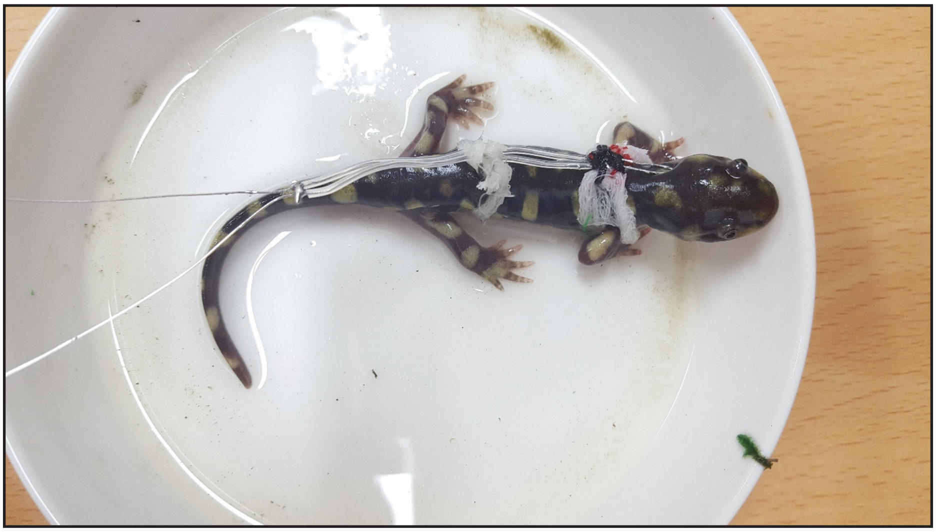

Figure 3. View from above the test Tiger Salamander (*Ambystoma tigrinum*) with an early version of the harness. Note that in this version the second soldering point is too low on the tail and the waist bands are not crossed over the rear legs of the individual. (Photographed by Kyungmin Kim).

resulted in a mean detection range of 190.33 ± 18.26 (mean ± SD) cm, a 2 cm loop resulted in a 219.42 ± 18.53 cm detection range, and a 3 cm loop resulted in a 240.83 ± 13.26 cm detection range. The detection range among the three loop sizes differed significantly ($F_{2,33}$ = 27.13, $P < 0.001$).

The second set of empirical tests looked at the impact of antennae leg length on the detection range. The shorter leg length (12 cm) resulted in a 90.67 ± 17.15 cm range; whereas, the 15 cm leg length resulted in a 123.83 ± 20.80 cm range, and the last leg length (25 cm) resulted in a 210.42 ± 14.47 cm detection range. The range variation among each of the leg lengths differed significantly ($F_{2,33}$ = 146.98, $P < 0.001$).

Discussion

We developed a harness that firmly attaches an HDF tag to the body of a salamander and cannot be easily removed by the salamander in either terrestrial or aquatic environments. Furthermore, our design incorporates silicone tubing around the two parallel wires that extend down the back of the salamander, and the tubing allows the dipole to work in conditions where other types of tags would fail. HDF tracking is limited by the penetration of waves produced by the emitters, and these waves do not penetrate water deeply. Accordingly, the immersion depth of the tag born by the animal tremendously decreases the effective detection range (see the Supplementary Materials from Borzée et al. 2016 for empirical measurements). Finally, contact between the antennae and the skin of the individual carrying the tag decreases the effective detection range. Furthermore, because of the bulk of the harness and tube-encased wires, a salamander wearing the harness in a confined area, such as a burrow, will possibly encounter physical blockages and could potentially die of dehydration or drowning before the gauze naturally degrades.

When designing the tag itself, the 3-cm loop produced a better detection range. However, the loop was above the head of the salamander, and prevented the individual from lifting its head. In contrast, we did not detect any negative impact for the two other loops on the behavior of the salamander. The length of the antennae legs was also of significant to the detection range. Long legs may not get stuck in the surrounding environment because of the mechanical elasticity of the tin-plated copper wires, but they will prevent an individual in a narrow environment from moving backwards.

Finally, the distress of the test salamander was obvious when held upside-down to stich the gauze bands. This procedure is time consuming, and is difficult with reluctant individuals, so we would recommend the use of a short-term anesthetic such as Tricaine mesylate/MS-222 to decrease the stress encountered by the animals. The invasiveness of subcutaneous implants is therefore a viable option, as they remove the risk of entanglement, but do require surgery for implantation. Therefore, we recommend the use of this methodology in very precise conditions only, for large sized individuals, or with the few species living in open habitats.

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Amaël Borzée is interested in the breeding behavior and conservation of amphibians. He is studying the causes of the endangered status of the Suweon Treefrog (*Dryophytes suweonensis*), including diverse topics such as behavioral ecology, phylogenetics, and conservation. His research is now focused on the same subjects but has expanded to include several anuran clades in North East Asia. (Photographed by Yi Yoonjung).

Ye Inn Kim research interests primarily involve variations in physiological traits in amphibians, mainly to understand how environmental threats such as climate change may impact their survival. (Photographed by Yi Yoonjung).

Kyungmin Kim is interested in the conservation of endangered species, especially mammals. She has been characterizing and analyzing roadkill and is creating prediction maps for Leopard Cats (*Prionailurus bengalensis euptilura*). She is also interested in habitat conservation for various species and taxa. (Photographed by Kyungmin Kim).

Yikweon Jang is a Biology Professor, a National Geographic Explorer, and a published author. Jang's research interests focus on the ecology and evolution of communication in insects, frogs, birds, and mammals. One significant component of his research is citizen science programs, specializing in spatio-temporal distributions of some of the iconic species in the Republic of Korea. The ultimate goals of Jang's research are wildlife conservation and harmonious coexistence of humans and animals. (Photographed by Yikweon Jang).