Ecological Engineering 95 (2016) 738–742

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Short communication

An anti-predation device to facilitate and secure the crossing of small mammals in motorway wildlife underpasses. (I) Lab tests of basic design features

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ARTICLE INFO

Article history:
Received 11 February 2016
Received in revised form 27 June 2016
Accepted 23 July 2016

Keywords:
Underpasses
Cricetus cricetus
Fragmentation
Conservation
Road
Highway

ABSTRACT

A great number of wildlife underpasses are used to mitigate the environmental impact of urbanization and road infrastructure expansion, thus restoring ecological connectivity. However, the simultaneous use of these structures by small mammals and their predators could result in increased predation rates in these passages or lead small mammals to avoid using them. This would be particularly harmful to small populations or threatened species such as the European hamster (*Cricetus cricetus*). To overcome this problem and to provide lateral escape opportunities along the length of the underpasses, we developed an anti-predation tube. We tested the features (shape and size) of this device under laboratory conditions and validated its use by captive European hamsters. Our results reveal that the optimal anti-predation tube has a diameter of 10 cm, a curved shape and lateral openings. This device will be tested under field conditions to validate its efficiency to protect small mammals using wildlife underpasses. If confirmed, this system could considerably improve crossing conditions in bigger tunnels and on bridges such as agricultural under- or overpasses, which have been unsuitable for small animals until now.

1. Introduction

The high demographic growth of human populations has produced soaring urbanization and road infrastructure development since the beginning of the 20th century (Seiler and Folkeson, 2006), causing substantial habitat loss. The development of the road infrastructure entails the accidental killing of animals by vehicles and causes fragmentation, leading to the isolation of wild populations, the loss of genetic diversity and border effects with a consequent repercussion on population dynamics and survival (Coffin, 2007; Frankham et al., 2002; Haddad et al., 2015). These negative effects are particularly harmful to endangered or small populations (Frankham et al., 2002; Jaeger and Fahrig, 2004), which are highly sensitive to environmental stochasticity (Courchamp et al., 1999; De Roos et al., 2003).

The European hamster (*Cricetus cricetus*) is one such species. It is critically endangered in Western-Europe (Villemey et al., 2013), and the French area of this species (*i.e.* in the Alsace Region) has decreased by 94% since 1972 to current levels of less than 1500 individuals (Reiners et al., 2014). The road transport network developed at an alarmingly high rate during the same period (Carsignol, 2006, 2005; Saussol and Pineau, 2007). In Alsace, a major motorway project is currently underway in one of the relict population core areas of this species (Dantec, 2014). In order to avoid the isolation of wild individuals and take mitigation and compensation measures for road construction, wildlife under- and overpasses have been built to restore connectivity in Alsace (DREAL, 2011; Gilbert-Norton et al., 2010; Saussol and Pineau, 2007). These structures – and other non-specific passages such as culverts, which are known to be suitable for the crossing of small mammals (Mata et al., 2008) – allow the dispersion and migration of a wide range of species (Carsignol, 2006; Forman et al., 2002; Mata et al., 2008). The simultaneous presence of prey species (*e.g.* small mammals such as rodents and shrews) and their predators (*i.e.* fox, cats, mustelids) in wildlife underpasses (Carsignol, 2005; Grilo et al., 2008; Little, 2003; Little et al., 2002; Mata et al., 2008) entails an

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http://dx.doi.org/10.1016/j.ecoleng.2016.07.012
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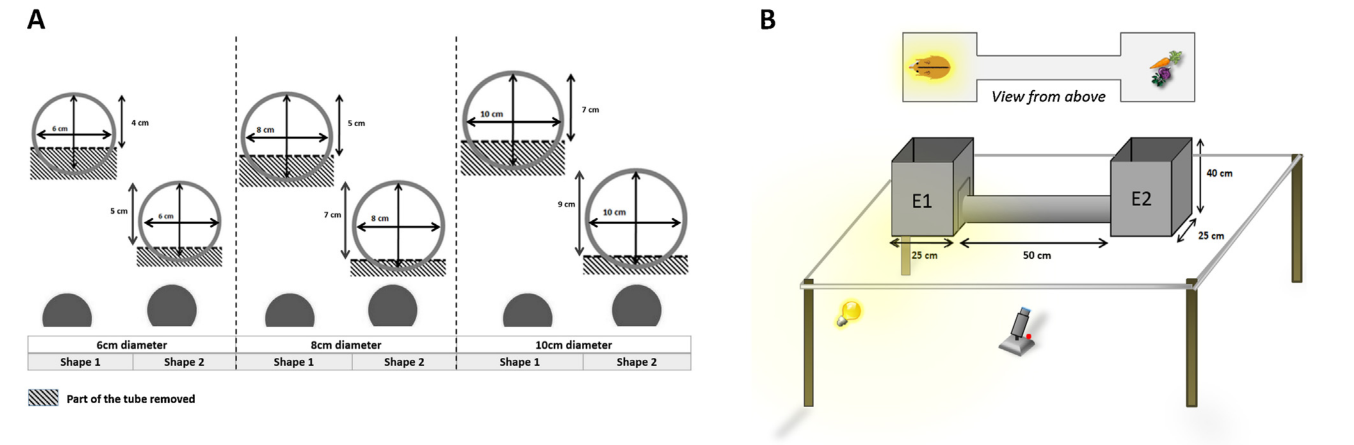

Fig. 1. Experimental design to validate the optimal shape and size of the APT. The three diameters and two shapes tested in Experiment 1 (6 combinations) are shown in **A**. The design in which each hamster was tested for the 6 combinations is shown in **B**.

increased predation risk for small mammals using these infrastructures. Consequently, small mammals may avoid these underpasses (Ruiz-Capillas et al., 2013) which might become potential ecological traps (Little, 2003). Although this idea has been widely debated (Little et al., 2002), particularly with regard to large mammals, very little is known regarding rodents. In the specific case of endangered species, the risk of possible predation cannot be ignored. A variety of road-crossing structures are available, and those targeting aquatic organisms and amphibians are apparently much more prevalent than those designed for terrestrial animals (Ward et al., 2015). More attention should thus be paid to providing suitable crossing structures for terrestrial animals. As indicated by Mata and collaborators (Mata et al., 2008), the adaptation or enrichment of culverts should not be ignored given the significance of these structures for certain species (such as badgers or small mammals) and their relatively low cost.

In this context, we developed a "sub-tunnel" type anti-predation device, *i.e.* a small tube to be placed inside the passages that mimics the natural galleries used by wild European hamsters. This article presents the tests carried out in captivity to determine the optimal features of this anti-predation tube (APT) and determine whether hamsters use it spontaneously. This is the preliminary step before field tests and the potential recommendation to implement this type of device on a large scale. The APT should ultimately enable hamsters and other small mammals to avoid or escape any predators they encounter in the passage by either using the APT for the entire crossing (avoidance) or by entering this tube through lateral openings when in danger in the underground passage (escape). This APT has been developed as part of a conservation program (LIFE+ Alister) for the European hamster in France which aims at restoring the connection of wild populations of the species in Alsace, France.

2. Material and methods

2.1. Animals and husbandry conditions

The experiment was performed on 18 unrelated captive European hamsters (9 males and 9 females). Males weighed on average 443 ± 139.9 g and females 352.8 ± 66.9 g. Individuals were housed in transparent Plexiglas cages (420*265*180 mm, D*W*H) and their environment was enriched with wood and shredded paper. Animals were provided with an *ad libitum* supply of water and food pellets (N° 105, from Safe, Augy, France). The experimental protocols followed EU Directive 2010/63/EU guidelines for animal experiments and the care and use of laboratory animals, and were approved by the Ethical Committee (CREMEAS) under agreement number 02015033110486252 (A PA FIS#397). 01.

2.2. Experiment 1: shape and size of the APT

The goal of this first experiment was to find the ideal size and shape of the APT. We aimed to create a device that would not affect the crossing of animals larger than hamsters, and would be inaccessible to relatively small predators. Consequently, it had to be as small as possible whilst allowing the crossing of small mammals of various sizes, including the European hamster, which is one of the largest rodents in France (Fenyk-Melody, 2012). The device should also be low cost and easy to clean for a widespread use in wildlife underpasses and culverts. European hamster galleries in the wild vary in shape, and diameters range from 4 to 10 cm (Marquet, 2014). We therefore tested two shapes and three diameters of plastic PVC tubes (Fig. 1A) using the device shown in Fig. 1B (based on the Chiaroscuro tests in rodents). This first experiment used unperforated 50 cm lengths of tube. A sample of 10 hamsters (5 males and 5 females) of varying corpulence (from 249 g to 608 g) was used for this experiment. Each hamster was randomly tested for the 6 combinations of tubes for 5 min in each tube (60 tests in total) and were never tested twice on the same day. Each subject was placed at one end of the tube (E1, see Fig. 1B) while appetizing food items (onions and carrots) were placed at the other end (E2, see Fig. 1B) to motivate the animals to cross the tube. The device was cleaned with ethanol after each trial. The experimental design was set up on a transparent table to enable filming during the tests, which were carried out in low light conditions (20W-light bulb) and at ambient temperature (19 °C ± 2 °C).

2.3. Experiment 2: spontaneous use of the APT

The goals of this second experiment were to test whether hamsters spontaneously used the APT and the lateral entrance/exits. Our APT prototype consisted of 2.78m-long sections of PVC tubing with a diameter of 10 cm (see 3.1 of Results section for further details). Holes of the same diameter were cut on alternate sides of the tube every 1 m to allow the lateral entrance/exit of individuals. The device was then placed in an artificial enclosure that reproduced the shape of a classic wildlife underpass (1 m wide × 0.40 m high × 3 m long; Fig. 2). Eight hamsters (4 males and 4 females) were randomly placed in the enclosure for habituation one day before the trials. Each individual was then randomly tested for 12 min in 3 conditions (see Fig. 2): left (**L** = the individual was placed in the enclosure, on the left of the anti-predation device), right

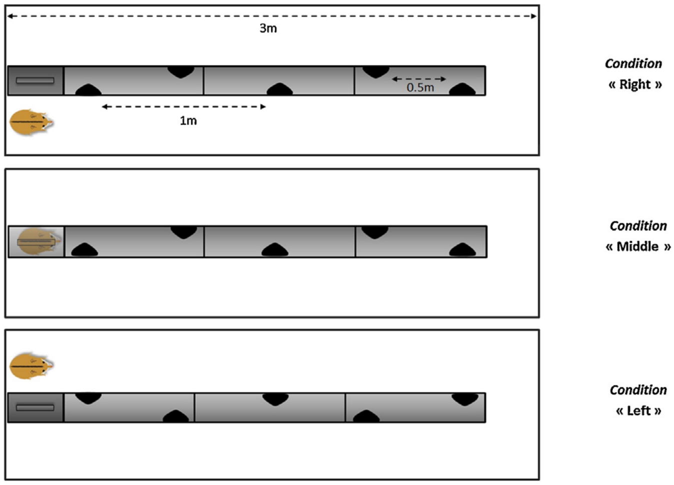

Fig. 2. Experimental design to validate the use of the APT by European hamsters (Experiment 2).

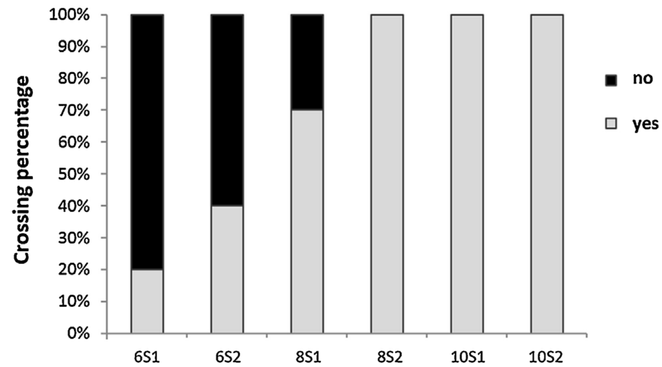

Fig. 3. Crossing percentage from end E1 to end E2 according to the diameter (6 cm, 8 cm and 10 cm) and the shape (S1 and S2) of the tube.

(**R** = the individual was placed on the right of the anti-predation device) and middle (**M** = the individual was placed inside the anti-predation device). After each trial, the enclosure and the device were cleaned with ethanol and the room was aired. Experiments were carried out and filmed under low light conditions and at an ambient temperature of 22 °C.

2.4. Data analyses

Four variables were considered when selecting the optimal form and diameter of the APT (test 1, Fig. 1), namely **(i)** the latency between the beginning of the test and the first arrival of the hamster at E2; **(ii)** the duration of the first crossing from E1 to E2 (time between the first entrance in the tube and the first exit in E2); **(iii)** the number of times individuals entered or exited the tube via E1 and E2 (representing the degree of use of the device) and **(iv)** the number of partial crossings from the end of the tube (*i.e.* only the head or the two front paws entered the tube, indicating a reluctance to cross). Variable **(ii)** was log-transformed to guarantee the normality of the residuals. Data were analyzed using a linear mixed model (LMM) composed of four fixed factors: the diameter and the shape of the tube, the sex of the individuals and the consumption of food at E2. The testing order (to control for possible habituation to the device) and the age of the individuals were included as covariates. As body mass is strongly correlated to the sex and the age of the individuals in this species (Fenyk-Melody, 2012), this variable was not included as a covariate in our models to avoid multicollinearity. We controlled for repeated measures on the same individual by including its identity as a random factor in our models.

When looking at the use of the APT by the hamsters (Test 2, Fig. 2), we considered 3 different variables: ($\mathbf{i}_2$) the time spent in the tube per hour, ($\mathbf{ii}_2$) the latency between the beginning of the test and the first entrance to the tube via a lateral opening (representing the speed of decision to use the device) and ($\mathbf{iii}_2$) the number of times an individual uses the lateral entrances in the tube per hour (representing the degree of use of the device). Variables ($\mathbf{i}_2$) and ($\mathbf{ii}_2$) were analyzed using GEE models (Generalized Estimation Equations for variables with residuals that did not follow a normal distribution; *i.e.* with binary responses or enumeration). The ($\mathbf{iii}_2$) variable was analyzed using a linear mixed model (LMM), using the identity of the individual as a random factor. Three fixed factors were included in these models, namely the test condition ("left", "right" and "middle"), the order of the tests and the sex of the individuals. For the "middle" condition (in which the hamster was placed inside the APT), the time the individual spent in the tube before the first exit was excluded from the analyses.

Normality was tested using a Kolmogorov-Smirnov test and variance homogeneity was checked using the Levene test. Multiple comparisons were analyzed via post-hoc LSD (least significant difference) testing. Model selections were carried out parsimoniously using an ascendant stepwise procedure combined with AICc verification (Akaike information criterion corrected for small samples). Analyses were conducted using IBM SPSS software (IBM SPSS Statistics for Windows, Version 21.0., IBM Corp., Armonk, NY, released 2012), and the significance threshold was set at $p < 0.05$.

3. Results

3.1. Shape and size of the APT (Experiment 1)

A total of 60 tests were carried out on 10 individuals (10 trials for each type of tube, Fig. 1). Hamsters only used the 6 cm diameter tube in 1/3 of the 20 tests (Fig. 3, 6S1 and 6S2). For subsequent analyses we therefore only considered tubes with diameters of 8 cm and 10 cm (*i.e.* 8S1, 8S2, 10S1 and 10S2 tubes). When the hamsters successfully crossed from E1 to E2, they did so without stopping in 98% of cases. Two females turned back, and this occurred four times in the 10 cm diameter tubes.

The diameter of the tube significantly affected **(i)** the latency between the beginning of the test and the first arrival of the hamster at E2 (Fig. 4A, $W = 269$, $p < 0.01$), which was significantly shorter in the 10 cm diameter tube. It significantly increased **(iii)** the number of times individuals entered or exited the tube via E1 and E2 (Fig. 4B, $F_{1,22} = 82.629$, $p < 0.01$). Finally, it also had an effect on **(iv)** the number of partial crossings (indicating a reluctance to cross), which was higher in the 8 cm diameter tube than in the 10 cm diameter tube (7.1 ± 1.9 and 5.3 ± 1.9 respectively; $F_{1,18} = 7.893$, $p = 0.012$).

The shape of the tube affected **(i)** the latency before the first arrival of the hamster at E2 ($F_{1,33} = 5.333$, $p = 0.027$), which was shorter in the S2 tube than in the S1 tube (66.9 ± 12.7 s and 111.1 ± 14.4 s respectively) and **(iii)** the number of times individuals entered or exited the tube via E1 and E2 (Fig. 4B; LMM, $F_{1,22} = 21.027$, $p < 0.01$). Finally, **(ii)** the duration of the first crossing from E1 to E2 was not affected by the diameter or the shape of the tube (LMM, $p > 0.05$). We found no effects of sex, age or testing order on the four variables (LMM, $p > 0.05$).

3.2. Spontaneous use of the APT and the lateral entrances/exits (Experiment 2)

A total of 24 trials were carried out on 8 individuals in this experiment. The eight individuals entered the tube in every trial, with one male refusing to enter the tube in two of its three trials. We

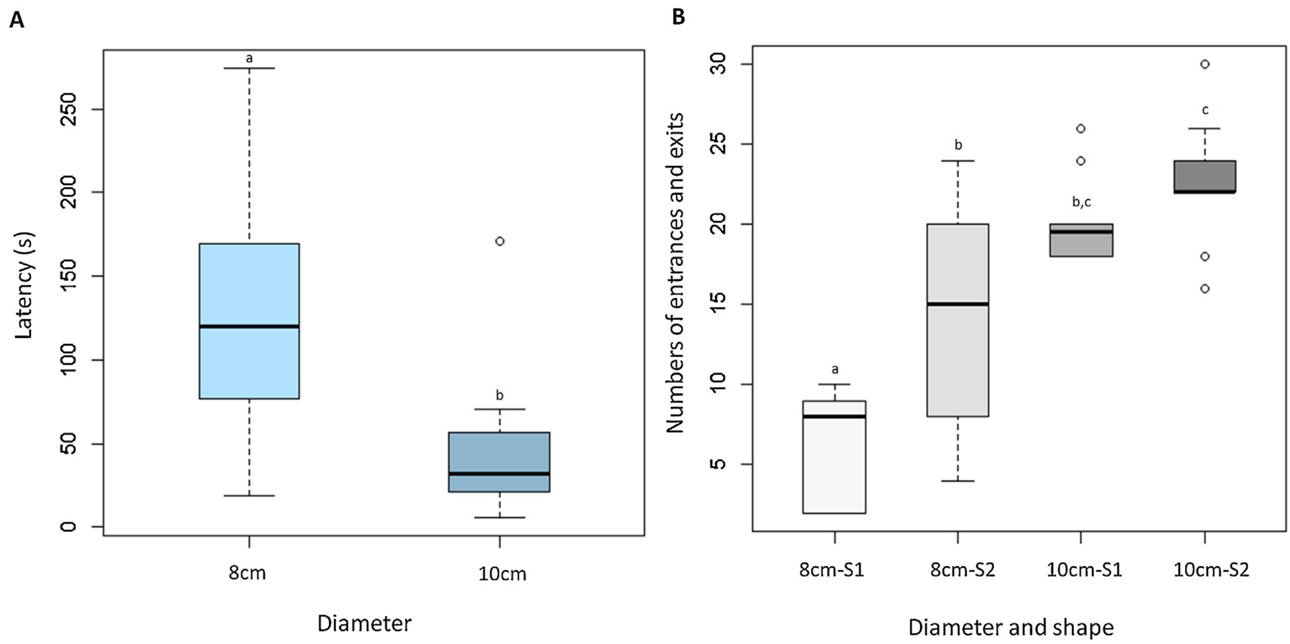

Fig. 4. Efficiency of the APT according to its diameter and shape (Experiment 1). **(A)** Latency between the beginning of the test and the first arrival of the hamster at E2 according to the diameter of the tube (8 cm and 10 cm) and **(B)** Number of times individuals entered or exited the tube via E1 and E2 according to the diameter (8 and 10 cm) and the shape (S1 and S2) of the tube. The different letters highlight significant differences between the groups ($p < 0.05$).

found inter-individual differences, with individuals entering the tube on average 351 ± 224 s after the start of the trial (total duration of 720s, see Section 2.3). Hamsters spent a total of 34 ± 32 s inside the tube (9% of the total test time) and made 8 ± 7 complete crossings between the tube and the artificial enclosure. We found an effect of sex on (**i**$_2$) the time spent in the tube per hour (GEE, $p = 0.035$), (**ii**$_2$) the latency between the beginning of the test and the first entrance to the tube via a lateral opening ($F_{1,6} = 29.128$, $p < 0.01$) and (**iii**$_2$) the number of times an individual uses the lateral entrances in the tube per hour (GEE, $p = 0.004$). Females spent more time inside the tube than males did (230 ± 133 s and 112.5 ± 133 s respectively), entered the tube more frequently (hourly frequency of 22.45 ± 7.4 s for females and 8.79 ± 2.5 s for males) and entered the tube sooner than males did (163.5 ± 49.7 s and 480.5 ± 50.6 s respectively for females and males). We found an effect of condition (M, R and L) on the number of times lateral entrances were used (GEE, $p < 0.01$): this number was significantly higher in the M condition than in the two others (Wilcoxon tests, $p < 0.05$).

4. Discussion

The results of these laboratory tests reveal that the 10S2 tube (*i.e.* diameter of 10 cm and curved shape) is the most suitable tube for the anti-predation device. Although the diameter of European hamsters galleries in the wild varies from 4 to 10 cm (Marquet, 2014), we found that 70% of the animals failed to enter the 6 cm diameter tubes, and 30% did not enter the 8S1 tube. Individuals that failed weighed more than 300 g and 400 g respectively. We can therefore conclude that only juveniles or small adults could use such small tubes. We also found that whatever the shape of the tube, individuals crossing those with a 10 cm diameter did so faster and more frequently than individuals using the 8 cm tubes. Regardless of the diameter, Shape 2 appears to be more appropriate than Shape 1, and increases the speed of the decision to cross and the frequency with which individuals use the tube.

As the European hamster is one of the biggest rodents in France (Fenyk-Melody, 2012), this tube could thus also be used by small rodents (*e.g.* voles, shrews...). However, the two smallest mustelids – the Stoat (*Mustela ermine)* and the least weasel (*Mustela nivalis*) – can enter galleries with diameters of less than 4 cm (Dayan and Simberloff, 1994; Gliwicz, 1988) and would therefore probably use this device as well. In this case, the APT would not entirely suppress predation pressure but would still reduce it by preventing the predation of small rodents by larger predators such as cats and foxes. Such cases of predation (*e.g.* domestic cats preying on up to 12 voles in one night) have recently been observed in monitored underpasses in the Alsace (*unpublished data*). We thus hypothesize that small mammals would globally be favored by the presence of the APT, which would allow them to avoid predators (even mustelids) by using the lateral holes to avoid any such predators in the passage. This hypothesis is currently being tested in captive and semi-natural conditions.

The second experiment reveals that all the individuals spontaneously used the device when placed in an artificial enclosure that mimicks the shape of a wildlife underpass. It was often used to cross from one side of the enclosure to the other. Results also reveal that females use the APT more than males and enter it more quickly. This could be explained by a difference in personality, as females are generally more anxious than males (Réale et al., 2007). They may therefore have sought refuge in a confined space such as the tube. It would be of interest to see whether this difference persists under natural conditions. Our next studies will investigate whether hamsters increase their use of the APT in presence of predation cues (*e.g.* proximity of predator urine or a cage containing a predator).

Following the results of our two experiments, the 10S2 tube has been selected to be placed in several wildlife underpasses and culverts in the Alsace (France). These devices will be monitored to validate the use of the APT by the European hamster and other small mammals. We will also investigate whether the use of the APT by hamsters in wildlife underpasses – which can be up to 50 m long in the Alsace – confirms the findings of this laboratory experiment. The length of the APT will be extended to one meter beyond the end of the main underground passages, with lateral exits, to reduce the risk of cats and foxes catching small mammals leaving the APT. We will also observe whether other small species use it to cross the passages.

5. Conclusion

This anti-predation tube is a potential tool to provide a specific passage facilitating the safe crossing of small animals within wildlife underpasses and culverts. It could also be used to enrich bigger passages (*e.g.* agricultural overpasses or wildlife bridges), that are currently unsuitable for small animals (Mata et al., 2008).

Acknowledgments

We are grateful to Pierre Uhlrich for making the device used to select the optimal shape and size of the APT and to Joanna Lignot for the copy editing. This work was supported by the LIFE + Biodiversity grant No. LIFE12 BIO/FR/000979 and the Ministère de l'Ecologie, du Développement durable et de l'Energie. The funders played no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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