



Using sentinel prey to assess predation pressure from terrestrial predators in water-filled tree holes

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Abstract. Tree-related microhabitats are important for forest biodiversity. Water-filled tree holes are one such microhabitat and can be abundant in temperate forests. The arthropod community in this microhabitat not only contribute to forest biodiversity but also provides food for terrestrial predators such as arthropods, small mammals and birds. The extent of the threat of attack from terrestrial predators on insect larvae in this microhabitat, however, is poorly known. To measure predation in this microhabitat, we produced fake prey resembling insect larvae using white plasticine and exposed them at the aquatic-terrestrial habitat interface. We recorded: (1) which predators attacked the fake larvae, (2) the predation probability on the fake larvae after two days and after two weeks and (3) whether predation probability on fake larvae differed between managed and unmanaged forest zones in one of the last primeval beech forests, the Uholka division of the Carpathian Biosphere Reserve in the Ukrainian Carpathians. By addressing these questions, we aimed to quantify the predation pressure of terrestrial predators on insect larvae in tree-holes. The probability that a fake larva in a tree hole was attacked by predators ranged between 25–58% (95% CI) after two days and between 76–96% (95% CI) after two weeks. Overall, the highest attack rates were recorded for small mammals, followed by arthropods and birds. Arthropods took longer to detect potential prey items than small mammals and birds, and they were the only group that showed significant differences in attack rates between forest zones (unmanaged > managed). This study revealed that sentinel prey might be a suitable method for measuring the predation pressure from terrestrial predators on insect larvae in water-filled tree holes.

INTRODUCTION

Tree-related microhabitats, such as cavities, tree injuries and exposed wood, crown deadwood, excrescences and fruiting bodies of saproxylic fungi, are important for maintaining biodiversity (Michel & Winter, 2009; Bütler et al., 2013; Müller et al., 2014; Larrieu et al., 2018). Given their importance, forest management strategies in Europe aim to increase the number and diversity of tree microhabitats in managed forests by retaining, or even creating artificially, a certain number of tree microhabitats per hectare (Brahma & Starre, 1976; Doerfler et al., 2017). Among tree microhabitat types, dendrotelmata (i.e. water-filled tree holes) can be abundant in unmanaged and managed temperate forests. For example, Gossner et al. (2016) found up to 56 tree holes per ha, differing widely in size, detritus amount and water chemistry, and consequently in insect abundance

and species richness. Depending on their exposure, water-filled tree holes retain water for different periods of time and show different degrees of buffering of their microclimatic conditions (Gossner, 2018).

Water-filled tree holes in temperate forests harbour a very specialised community of invertebrates, including aquatic insect larvae of many flies and midges and one beetle family (Kitching, 2000). Such insect larvae contribute substantially to forest biodiversity, for example as food for higher trophic levels, including terrestrial predators such as arthropods, small mammals and birds. In this way, they might substantially contribute to trophic chains and nutrient cycling in forest ecosystems. Despite its potential importance, our knowledge of the trophic interaction at the aquatic-terrestrial habitat interface is still limited because of the lack of suitable tools for monitoring.

Sentinel prey have been used to measure predation pressure in different ecosystems and to determine global gradients in predation (Leidinger et al., 2017; Roslin et al., 2017; Romero et al., 2018; Meyer et al., 2019; Zvereva et al., 2019). We recorded attacks on fake larvae as a proxy for predation pressure by terrestrial predators in water-filled tree holes. We created plasticine models resembling insect larvae and exposed them at the aquatic-terrestrial habitat interface, thereby testing the suitability of this method for quantifying predation pressure in water-filled tree holes. Our aims were: (1) to identify the predators of insect larvae in water-filled tree holes, (2) to quantify predation probability on fake larvae after two days and after two weeks and (3) to determine whether predation probability on fake larvae differed between two differently managed forest zones. We expected predator abundance and thus predation probability to be higher in unmanaged compared to the managed part of the forest. Furthermore, we expected that birds and mammals detect potential prey quickly and that their attack rates decrease with time following a learning curve. Prey detection by arthropods might take longer and no learning effect is expected.

MATERIALS AND METHODS

Study site

This study was conducted in the Uholka-Shiroky Luh forest, part of the Carpathian Biosphere Reserve in the Ukrainian Carpathians (48.2695°N, 23.6207°E; 700–800 m a.s.l.) between 4 and 21 September 2018. The forest covers an area of 8800 ha and is therefore the largest contiguous primeval beech forest (*Fagus sylvatica* L.) worldwide. It was designated a UNESCO World Heritage Site in 1992. The Uholka-Shiroky Luh forest is divided into three zones (Fig. 1), based on the Seville Strategy (UNESCO 1996) and Madrid Action Plan (UNESCO/MAB 2008) for biosphere reserves. Within the core zone natural processes occur without any intervention and all human activity there is restricted. In the buffer zone some conservation measures for restoring and protecting the natural ecosystem are allowed. In the anthropogenic zone traditional land management is practiced (Commarmot et al., 2013). In both the core (10 ha permanent plots of the Swiss Federal Institute for Forest, Snow and Landscape Research WSL) and the buffer zone, seven water-filled pan holes (lined with bark, without contact with wood) at ground level [< 2 m height, according to the definition of Kitching (1971)] were chosen on different trees within an area of approx. 1 ha. Tree and tree-hole characteristics did not differ significantly between the core and the buffer zone (Fig. S1, Table S1). The distance between trees was similar in the core (mean \pm SE: 88 ± 6 m) and buffer zones (91 ± 7 m). The mean distance between protection zones was 1222 ± 8 m.

Sampling of insect larvae and assessment of predation

In each of the tree holes selected, one water sample of 15 ml was taken to assess the abundance and species richness of insect larvae (for details, see Gossner, 2018). Subsequently, five fake larvae made of non-toxic white plasticine (Noris Club 8421-0; STAEDTLER, Mars Deutschland GmbH, Nuernberg, Germany) were evenly distributed across the water surface of each selected tree hole. The fake larvae were $1.5 \text{ cm} \times 0.5 \text{ cm}$ in size, and modelled by hand around a black thread which was attached to tree bark with black drawing pins (Fig. 2). In addition, tree diameter at breast height was measured and tree-hole physical characteristics were assessed, namely: height above ground, size of the entrance

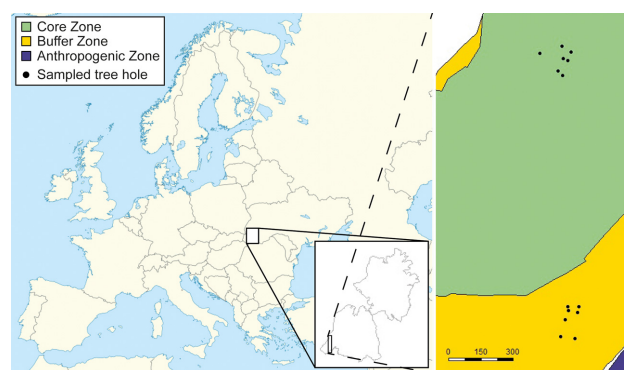


Fig. 1. Map showing the locations of the water-filled tree holes studied in the core and buffer zones in the Uholka-Shiroky Luh forest, Ukraine.

and maximum water volume calculated based on measurements of the length (largest diameter), width (smallest diameter) and depth of the hole, thus approximating it to a cone.

Predation probability was assessed twice in order to test whether predation by different predator groups changed over time. Fake larvae were retrieved for the first time on 6 September 2018, two days after installation (4 September 2018) and for the second time after two weeks (exposure time 6–21 September 2018). All fake larvae were replaced with new models between the two measurements. Thus, in total, 140 fake prey were exposed to predators during this study (2 management zones \times 7 trees \times 5 fake larvae \times 2 time periods). After retrieval, all fake larvae were stored separately in small, labelled plastic boxes and subsequently inspected for marks made by predators (following the examples given in Low et al., 2014; Meyer et al., 2017; Castagneyrol et al., 2018) using magnifying lenses and stereo microscopes in a laboratory. Predation probability was calculated as the percentage of fake larvae with marks made by predators (overall and by identified groups of predators, i.e. small mammals, birds and arthropods). Marks caused by molluscs were excluded from further analyses, as molluscs are rarely predators of insect larvae (Meyer et al., 2019).

The insect larvae (and pupae) in the 15 ml samples of water were identified to species or morphospecies level using a stereo microscope and identification keys (for details, see Gossner, 2018).

In addition, a 50 ml sample of water was taken from 6 tree holes (3 in the core zone and 3 in the buffer zone) and divided into 10 subsamples of 5 ml to determine whether the number of species and diversity increases as the size of the subsample increases. This approach was preferred over taking 10 5 ml samples because it is more likely that more species will be collected in one large sample than in several small ones. Thus, our approach was expected to better reflect the differences between samples of different volumes.

Statistical analyses

All analyses were done using R version 3.6.0 (R Core Team, 2019).

Linear models were used to test for differences in tree-hole structural characteristics between the core and the buffer zone. Response variables were either log- (height, estimated maximum volume) or square-root- (size of entrance) transformed to meet model assumptions.

Linear models were also used to determine the effects of forest zone (core vs. buffer zone) and tree-hole physical characteristics (height above ground, size of entrance, estimated maximum water

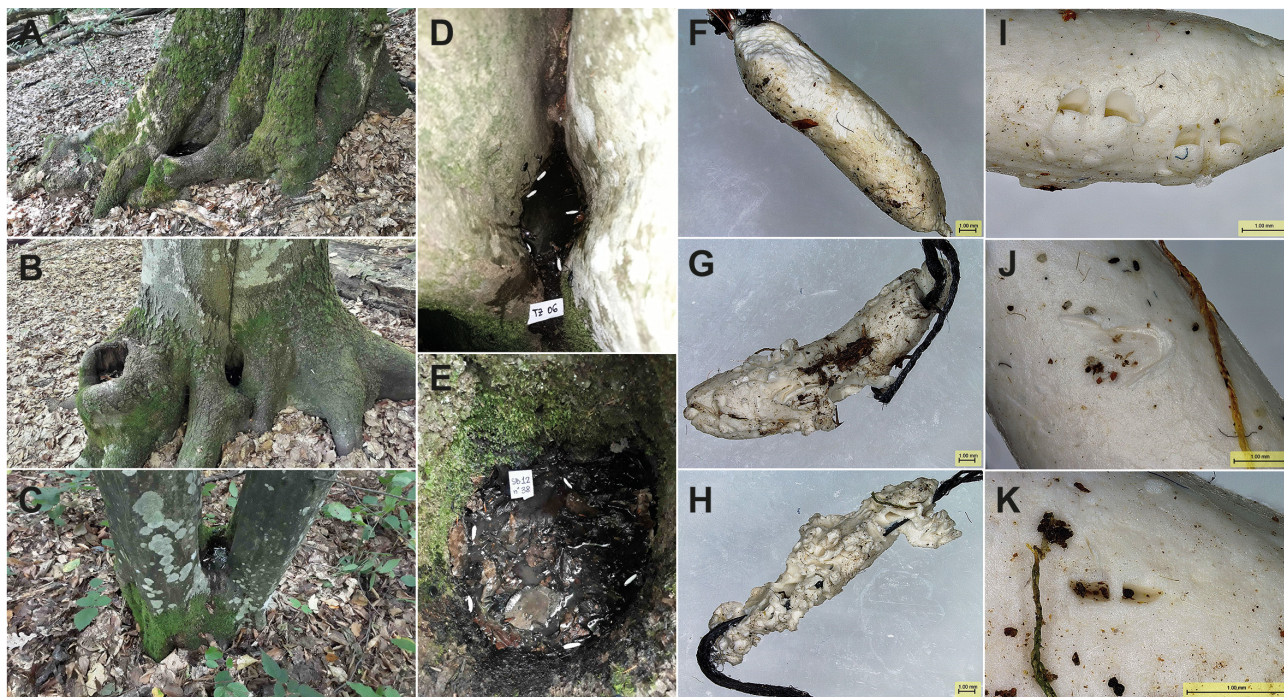


Fig. 2. Examples of water-filled tree holes and marks made by the predators on the model larvae in the present study: Pan tree holes in buttress roots of *Fagus sylvatica* in the core zone (A, B) and a stem fork of *Carpinus betulus* in the transition zone (C). Examples of pan tree holes with fake larvae made of white plasticine (D, E). Marks made by predators on fake larvae (pictures taken with Leica DVM6 Digital microscope): Rasping marks of molluscs (F) and deep marks, mainly made by small mammals (G, H). Close-up view of marks made by small mammals (I), birds (J) and arthropods (K).

volume) on the abundance and species richness of tree-hole insect larval communities. In the species richness model, the number of individuals sampled was included as an additional predictor. The number of individuals was log-transformed prior to all analyses. All quantitative predictor variables were scaled to zero mean and unit variance using the *deconstand* function in the ‘vegan’ package (Oksanen et al., 2019).

To determine the effects of forest zone (core vs. buffer zone) and tree-hole physical characteristics on percentage predation (probability of attack) of the five fake larvae per tree hole, generalised mixed effects models with a binomial error distribution and logit link function were applied using the ‘lmerTest’ package (Kuznetsova et al., 2017). The same predictor variables as for the species richness model were used, and the percentage of fake larvae attacked (function “*cbind* (number of larvae attacked, number of larvae not attacked)”) was the response variable. An observation-level random intercept term (TreeID) was also included to account for overdispersion in the models (Harrison, 2015). Model-derived probability estimates (LS-means \pm 95% CI) were calculated using the ‘emmeans’ package (Lenth, 2020).

To evaluate how species and diversity increases as the number of individuals sampled and size of the subsample increase, the ‘diversity accumulation curve’ framework was used (Chao et al., 2014). This approach extends methods for rarefaction and extrapolation of species richness to higher-order Hill numbers (Hill, 1973; Jost, 2006). It further allows estimation of sample completeness (Chao & Jost, 2012) and therefore the sample coverage-based estimation. A bootstrapping method was used to construct confidence intervals of the Hill numbers (Colwell et al., 2012). Calculations were done using the ‘iNEXT’ package (Hsieh et al., 2014). Sample-based estimations were derived from dependent samples and thus need to be interpreted with caution.

RESULTS

Abundance and species richness of insect larvae

In total, we collected 45 larvae of 10 insect species in the 15 ml samples from the 14 tree holes. *Metriocnemus cavicola* (13 ind.; Diptera: Chironomidae) and *Dasyhelea* sp. (13 ind.; Diptera: Ceratopogonidae) were the most abundant species, followed by *Prionocyphon serricornis* (8 ind.; Coleoptera: Scirtidae), an unidentified Scirtidae species (2 ind.), single individuals of *Aedes geniculatus* and *Anopheles plumbeus* (both Diptera: Culicidae) and of Syrphidae sp., Psychodidae sp. and Diptera sp. (all Diptera), and two unidentifiable larvae. On average 3.21 ± 0.87 (mean \pm SE; range 0–13) individuals and 1.71 ± 0.27 (range 0–3) species were recorded in the 15 ml samples ($n = 14$ tree holes).

Species accumulation curves based on the six 50 ml samples revealed that 15 ml samples are sufficient for representative sampling of the dominant and frequent species in the communities (curves of $q = 1$ and $q = 2$ approached an asymptote; Fig. S2). However, rare species might be under represented ($q = 0$; Fig. S2). Individual-based estimations showed that five individuals in a sample might be sufficient to reliably estimate the diversity and frequency of dominant and frequent species, while more individuals are needed to reliably estimate the number of rare species (Fig. S3).

While the number of insect larvae in the tree holes did not differ between the core and buffer zone, a significantly higher species richness was recorded in the core zone (Fig. 3, Table 1). The physical characteristics of the tree-holes

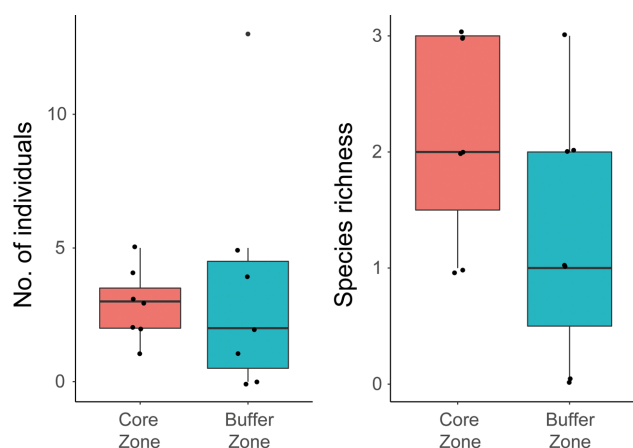


Fig. 3. Differences in the number of individuals and species per 15 ml sample from tree holes in the core and buffer zones in the Uholka-Shiroky Luh forest. Medians (thick line), 25%–75% quantiles (Box) and min–max values (Whiskers) excluding the outliers (points), are shown.

did not affect the number of larvae or the species richness. Species richness was positively associated with the number of individuals.

Probability of predation on fake larvae

Overall, 41% of the fake larvae were attacked after two days and 84% after two weeks. In each tree hole, the probability that a fake larva was attacked by predators ranged between 25–58% (95% CI across protection zones; mean \pm SE $40 \pm 9\%$, $n = 14$ tree holes) after two days and between 76–96% after two weeks ($89 \pm 5\%$; $n = 14$) (Fig. 4). After two days, the highest predation probabilities were recorded for small mammal predators ($22 \pm 9\%$; mean probability \pm SE across protection zones; $n = 7$ tree holes), followed by birds ($8 \pm 3\%$; $n = 7$) and then arthropods ($1 \pm 1\%$; $n = 7$). After two weeks, small mammals ($67 \pm 11\%$; $n = 7$) were still the most important, followed by arthropods ($53 \pm 7\%$; $n = 7$) and birds ($28 \pm 7\%$; $n = 7$). Furthermore, rasping marks made by molluscs were recorded on 19% of all fake larvae after two weeks, while no such marks were recorded after two days.

Predation probabilities by all predator groups increased significantly with time of exposure. In addition, a higher percentage of fake larvae was attacked by arthropods in the core compared with the buffer zone (Table 2, Fig. 4).

Neither small mammals nor birds followed the same trend. Predation probabilities by birds were higher for tree holes high above the ground and those with small entrances (Table 2). None of the other physical characteristics of tree holes affected predation rates.

DISCUSSION

This study revealed that a high percentage of model larvae exposed in the aquatic-terrestrial habitat interface of water-filled tree holes were attacked by small mammals, birds and arthropods. Predation probabilities by all predator groups increased with time, but a substantial percentage of fake larvae were attacked by small mammals and birds already after two days, whereas arthropod attacks occurred later. While there was a higher probability of predation by arthropods in the core zone compared with the buffer zone, no differences between zones were recorded for predation by small mammals or birds. Moreover, the probability of predation by birds was positively affected by the height of the tree holes and negatively by the size of the tree hole entrance.

Using fake larvae to study predation pressure in water-filled tree holes

We recorded an average percentage predation of 40% after two days and 89% after two weeks. Arthropods mostly attacked fake larvae later than two days of exposure and for some tree holes total predation was 100% after two weeks. This indicates that fake larvae should be exposed for more than two days to capture arthropod predation, but less than two weeks to avoid 100% attack, which would make the comparisons between habitats or treatments impossible.

Previous studies using fake caterpillars made of plasticine have mainly been conducted on the ground (Leidinger et al., 2017; Meyer et al., 2019), on seedlings and shrubs near the ground (Roslin et al., 2017) or on tree branches (Mantyla et al., 2008; Castagneyrol et al., 2020). The percentage predation recorded in this study are within the range of those reported for grasslands (63% attacked after three days; Meyer et al., 2019), on oak trees (between 26 and 33% per 15 days; Castagneyrol et al., 2020) and across ecosystems globally (up to 35% per day, Roslin et al., 2017).

We recorded differences in percentage predation between predatory groups. Predation by arthropods might be lower

Table 1. Results of the linear models of the effects of forest zone, abundance of insect larvae and tree and tree-hole characteristics on the number of individuals and species richness per 15 ml water sampled from the tree holes studied. Values in bold indicate statistical significance ($p < 0.05$).

| | Abundance | | | Species richness | | |
|----------------------------------|-----------|--------|-------|------------------|---------------|--------------|
| | df | t | p | df | t | p |
| Intercept | | 4.183 | 0.002 | | 5.700 | <0.001 |
| Abundance of insect larvae (log) | — | — | — | 1 | 2.538 | 0.035 |
| Height of tree hole | 1 | 0.460 | 0.657 | 1 | 0.870 | 0.409 |
| Estimated maximum volume | 1 | −0.928 | 0.378 | 1 | −1.446 | 0.186 |
| Size of the opening to tree hole | 1 | 0.761 | 0.466 | 1 | 1.228 | 0.254 |
| Forest zone (buffer vs. core) | 1 | −0.717 | 0.492 | 1 | −2.615 | 0.031 |
| Residuals | 9 | | | 8 | | |
| Adjusted R-squared | −0.285 | | | 0.403 | | |

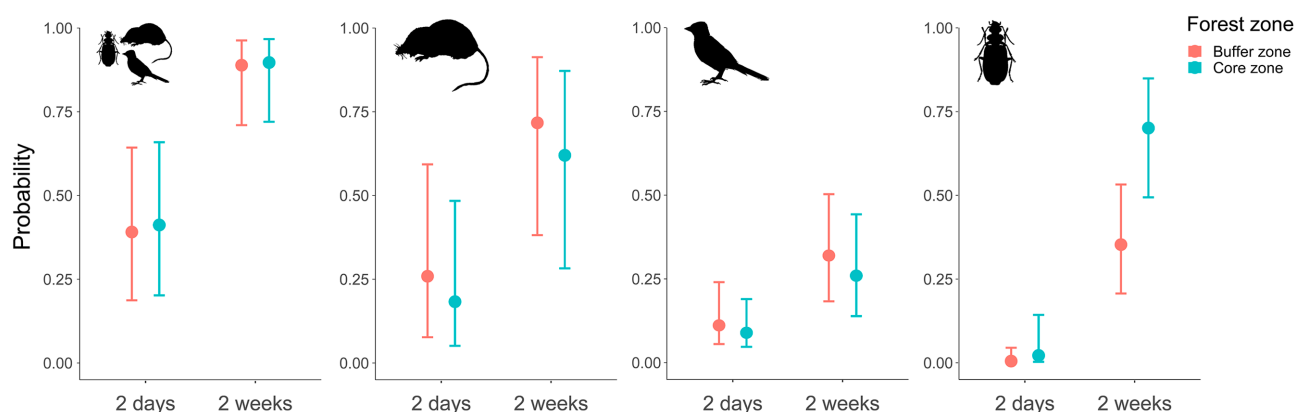


Fig. 4. Predation probabilities of fake larvae by all predators, small mammals, birds and arthropods in tree holes in the core and buffer zones in the Uholka-Schiroki Luh forest. Model-derived probability estimates are shown (LS-means \pm 95% CI, back-transformed from the logit scale to the original probability scale).

than that by vertebrates because they forage for prey differently. While vertebrates mostly search for prey visually (Church et al., 1998; Cronin et al., 2014), arthropods such as carabids often use chemical cues (Wheater, 1989; Kiely et al., 1996). These differences in prey recognition might explain the longer detection time recorded for arthropods (see above). In addition, different foraging strategies (i.e. preferences for foraging in particular strata) and life cycles might have affected the differences in predation recorded between predatory groups (see below). Along with traces of predation left by small mammals, birds and arthropods, we recorded radula marks left by molluscs on 19% of the model larvae. This is reported in several other studies (Low et al., 2014; Ferrante et al., 2017a; Hertzog et al., 2017; Meyer et al., 2017, 2019). Although molluscs might occasionally be predators (often of other molluscs), in this study we considered molluscs to be opportunistic scavengers, as suggested by Meyer et al. (2019). Probably they are attracted by the plasticine as a potential food resource.

In several previous studies, green-coloured models were used to mimic a “general caterpillar” (Lövei & Ferrante,

2017) as green caterpillars might be perceived by predators as palatable and undefended prey (Howe et al., 2009). However, attack rates on differently coloured prey might differ greatly due to differences in luminance and predators’ colour perception (e.g. tetrachromacy in birds, Vorobyev et al., 1998). In addition, the effect might depend on the biome, habitat and type of predator (Zvereva et al., 2019). We used white-coloured fake larvae to mimic the dominant prey in tree holes, which are white to yellowish insect larvae. “Real” predation rates are generally difficult to assess in nature, but reliable results are reported in other studies using green sentinel prey. In some studies, predation rates on sentinel prey did not differ from predation on real prey (Ferrante et al., 2017b), but in other cases predation rates on sentinel prey were lower (Hertzog et al., 2017; Lövei & Ferrante, 2017). The effects of environment, such as land use or altered plant species diversity (Hertzog et al., 2017), on percentage predation are well detected by using sentinel prey. Moreover, predation assessed by fake caterpillars (Mansion-Vaquié et al., 2017; Meyer et al., 2019) and aphid cards (Boetzel et al., 2020) is closely associated

Table 2. Results of the generalised linear mixed effects models (binomial error distribution) of the effects of abundance of insect larvae, tree-hole characteristics, time and forest zone on the predation probability of fake larvae, in total and by predator group (small mammals, birds, arthropods). Values in bold indicate statistical significant ($p < 0.05$) and values in bold italics marginally non-significant ($p < 0.10$) effects.

| | | Intercept | Insect abundance | Height of tree hole | Size of entrance to tree hole | Time | Forest zone |
|------------------|----|------------------|------------------|---------------------|-------------------------------|------------------|--------------|
| Total | df | | 1 | 1 | 1 | 1 | 1 |
| | z | −0.683 | −0.176 | −0.348 | −1.570 | 4.931 | −0.115 |
| | p | 0.494 | 0.860 | 0.728 | 0.116 | <0.001 | 0.908 |
| Effect direction | | | | | | 2w>2d | |
| Mammals | df | | 1 | 1 | 1 | 1 | 1 |
| | z | −2.054 | −0.232 | −0.959 | −0.860 | 3.894 | 0.437 |
| | p | 0.040 | 0.817 | 0.338 | 0.390 | <0.001 | 0.662 |
| Effect direction | | | | | | 2w>2d | |
| Birds | df | | 1 | 1 | 1 | 1 | 1 |
| | z | −4.848 | −0.964 | 1.900 | −2.077 | 3.004 | 0.585 |
| | p | <0.001 | 0.335 | 0.057 | 0.038 | 0.003 | 0.559 |
| Effect direction | | | | + | − | 2w>2d | |
| Arthropods | df | | 1 | 1 | 1 | 1 | 1 |
| | z | −3.711 | −0.874 | −0.798 | −0.799 | 4.335 | −2.377 |
| | p | <0.001 | 0.382 | 0.425 | 0.424 | <0.001 | 0.017 |
| Effect direction | | | | | | 2w>2d | Core>Buffer |

with the abundance of predatory ground beetles. For our system we conclude, that fake larvae are suitable for detecting differences in predation in tree-related microhabitats in relation to management and microhabitat characteristics. There are, however, limitations to the use of sentinel prey. For example, estimation of the overall biocontrol potential in different agricultural systems might be biased when sentinel prey is used (Zou et al., 2017).

In a previous study using camera traps installed above water-filled tree holes in beech and conifer forests in two regions of Germany, we recorded 24 species (15 birds, 8 mammals, 1 tree frog) using the tree holes as a source of water or food (Roberts et al., unpubl.), which confirms the importance of water-filled tree holes for terrestrial predators. The time it takes for insect larvae to complete their development in water-filled tree holes depends strongly on the microclimate, but is most likely around two months (Gossner, 2018). Thus, these species are very vulnerable to predation during the larval stage and thus predation by terrestrial predators might significantly affect their abundance. Future studies should aim to gain a more detailed understanding of how the predation rates recorded with fake larvae compares with natural predation detected using camera traps. Such studies would also enable comparisons of predation rates and the identification of the predators of model and real larvae.

What affects predation rates by terrestrial predators on the inhabitants of water-filled tree holes?

We recorded a higher species richness of insect larvae in water-filled tree holes and a higher percentage predation by arthropods in the core zone than in the buffer zone. This indicates that insect larvae in water-filled tree holes are negatively affected by management as is reported in previous studies (Gossner et al., 2016; Petermann et al., 2016; Petermann et al., 2020). Arthropod predators might be less abundant in the buffer zone due to lower resource availability. Ground beetles are important terrestrial predators of insect larvae developing in water-filled tree holes near the ground (pers. observ.). Less mobile, ground-active arthropod predators, such as many ground beetles (Pohl et al., 2007) might be less abundant and diverse in the buffer zone. Generally, management negatively affects ground-dwelling predators in forests (Paillet et al., 2010), although contrasting results are reported for historically intensively used landscapes in Central Europe (Lange et al., 2014). In the region studied the abundance of ground beetles is lower in managed compared to unmanaged forests (Chumak et al., 2015), most likely because of a lower abundance of prey. Many arthropod predators, such as the majority of ground beetles, are generalist predators (Lövei & Sunderland, 1996), and are thus unlikely to specialize on prey inhabiting water-filled tree holes. Thus, because of a higher availability of prey outside tree holes and an associated smaller radius of activity, they are likely to be less efficient at finding artificial prey in the buffer zone. In contrast to arthropods, small mammals and birds did not differ in their

predation rates in different protection zones. More mobile species, such as birds and mammals, are likely to forage in both zones and be more efficient at detecting new sources of prey. However, there might be a turnover of species that have similar predation efficiencies between zones. As we focused on the predation attempts on fake larvae and did not simultaneously study the abundance of terrestrial predators in the two zones, the mechanisms underlying the observed patterns remain unclear. More studies are needed to test whether terrestrial predators that forage near the ground in the beech forest area in the Carpathian region are generally negatively affected by management.

The probability of predation by birds increased with tree hole height and decreased with the size of the entrance to tree holes. Previous studies using green fake caterpillars report generally low predation rates by birds on the ground (see, e.g. Meyer et al., 2019), except in meadows after mowing (Solovyeva, 2015), while predation by birds is generally high in trees (Castagneyrol et al., 2018). This finding contrasts with that reported for other groups of predators, such as small mammals and arthropods, for which high predation rates are reported on the ground (Lövei & Ferrante, 2017; Boetzel et al., 2020) and in our own study. Thus, the vertical change recorded in the importance of different groups of terrestrial predators in terms of the predation of larvae in water-filled tree holes is consistent with the patterns reported in other forest habitats. In general, foraging by birds in hardwood forests depends on the abundance and type of prey, the structure and characteristics of the trees and therefore the detectability and accessibility of prey. Moreover, foraging by birds is related to the morphological and behavioural abilities of different species of birds to perceive and capture prey (Holmes & Schultz, 1988). Adamík (2004) studied the foraging ecology of two bark foraging passerine bird species, the nuthatch (*Sitta europaea*) and the Eurasian Treecreeper (*Certhia familiaris*), in an old-growth European beech dominated temperate forest in Slovakia and found that these birds preferred to forage on the trunk and larger branches. Both species are predators of the inhabitants of water-filled tree holes as reported in Germany (Roberts et al., unpubl.). This indicates that tree holes well above ground level are more likely to be detected by these birds. Thus, the higher percentage predation by birds of the inhabitants of tree holes high up on trees recorded in our study is most likely linked to the foraging behaviour of birds. The decrease in predation rates with increase in size of the entrance to the tree holes is surprising and contrasts with the visitation rates of birds reported in a study in Germany using camera traps (Roberts et al., unpubl.). Tree holes with large openings are more likely to be discovered by birds and contain more natural larvae (Gossner et al., 2016; Petermann et al., 2020). Our results might indicate a methodological issue, as fake larvae were more widely distributed over the water surface in large holes (Fig. 2). This might decrease the probability of a single fake larva being attacked by birds. However, this would also be the case for small mammals and arthropods and we did not record such an effect for them.

CONCLUSIONS

This is the first study in which sentinel prey have been used for studying predation on the inhabitants of tree-related microhabitats (Larrieu et al., 2018). To achieve a more detailed understanding of predation in water-filled tree holes, future studies should test: (1) how well the predation rates on fake larvae reflect natural predation; (2) whether predation is due to the opportunistic behaviour of predators or of species that are specialist feeders on the inhabitants of these habitats; (3) how important the availability and diversity of food items in water-filled tree holes is for predation by terrestrial predators; and (4) under what conditions water-filled tree holes provide an important foraging microhabitat in forests. To address these questions new methods should be developed for conducting experiments using artificial and natural prey under different environmental conditions. In addition, valuable information on the identity of the attackers can be obtained by using camera traps and their salivary DNA left on artificial prey, as recently suggested by Rößler et al. (2018). These new techniques could form the basis for a standard and rapid assessment of species interactions in tree-related microhabitats.

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AUTHOR CONTRIBUTIONS. MMG conceived the study and conducted the field and laboratory work, together with EG, VD, MJ and MY. MMG did the analyses, wrote the first draft and finalised the manuscript. EG and MJ commented on the manuscript.

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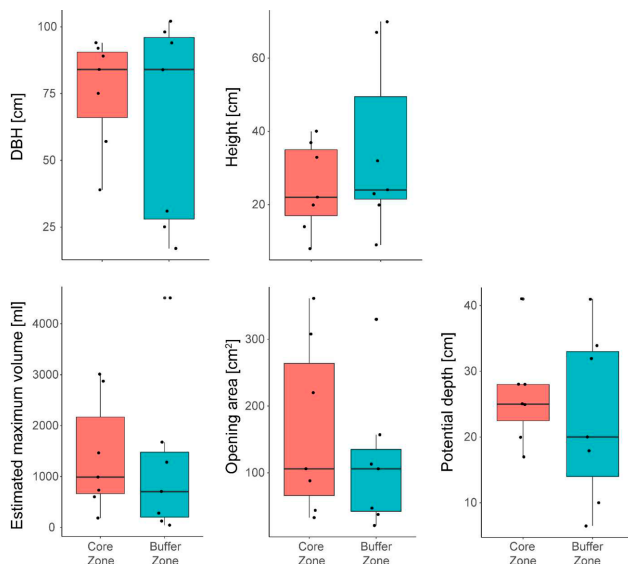


Fig. S1. Differences in physical characteristics of trees and tree holes sampled in the core and buffer zones of the Uholka-Shiroky Luh forest. No characteristics differed significantly between the two zones (see Table S1).

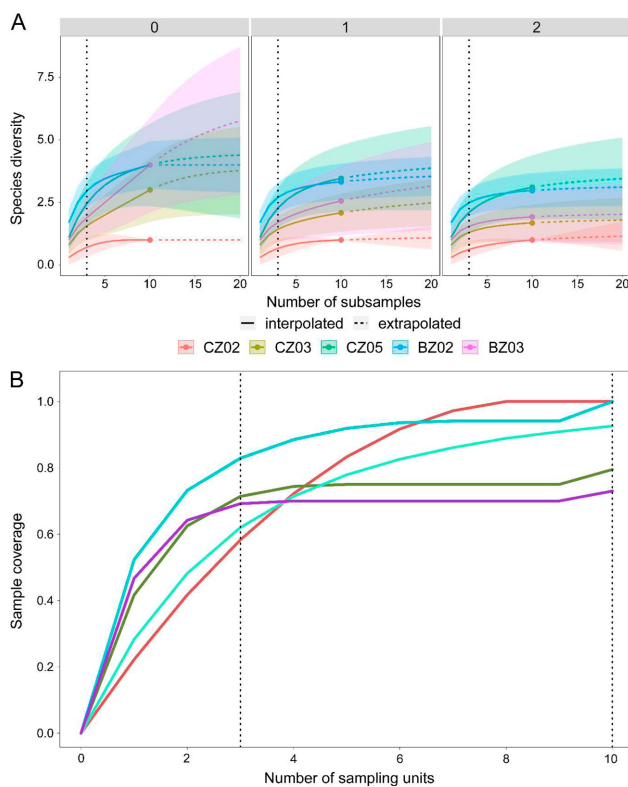


Fig. S2. A – Sample-based rarefaction and extrapolation to 20 times 5 ml subsamples (twice the sample size) including 95% confidence intervals, obtained by bootstrapping based on 200 replications. Panels show diversity quantified in terms of Hill-numbers 0, 1 and 2 (rarefaction – solid line, extrapolation – dashed line). In six tree holes (three in the core zone CZ, three in the buffer zone BZ), 50 ml water samples were taken, following the method described in Gossner (2018), and divided into 10 times 5 ml subsamples. One tree hole in the buffer zone was excluded from the analyses because the 50 ml sample did not contain any insect larvae. B – Estimated sample coverage with an increasing number of 5 ml subsamples considered (total = 10 times 5 ml). Please note that these analyses are based on non-independent samples and thus have to be interpreted with caution.

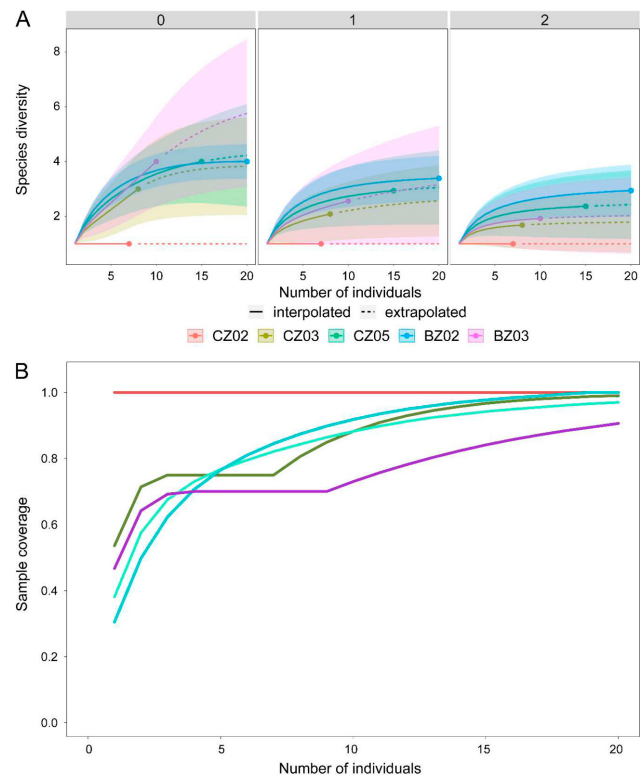


Fig. S3. A – Individual-based rarefaction and extrapolation to 20 individuals (maximum sample size) including 95% confidence intervals, obtained by bootstrapping based on 200 replications. Panels show diversity quantified in terms of Hill-numbers 0, 1 and 2 (rarefaction – solid line, extrapolation – dashed line). The analyses are based on the species abundances per 50 ml sample per tree hole, with three trees from the buffer zone (BZ) and three from the core zone (CZ). One tree hole in the buffer zone was excluded because the 50 ml sample did not contain any insect larvae. B – Estimated sample coverage with an increasing number of individuals sampled.

Table S1. Linear model results for differences in characteristics of trees and tree holes sampled in the core and buffer zones of the Uholka-Shiroky Luh forest. Negative estimates indicate higher values in the core zone and positive estimates higher values in the buffer zone. Transformations of variables applied prior to analyses are given in brackets.

| Characteristic (buffer zone vs. core zone) | df | Estimate | t-value | p-value |
|--|-------|----------|---------|---------|
| Tree DBH | 1, 12 | –11.290 | –0.689 | 0.504 |
| Tree-hole height (log) | 1, 12 | 0.261 | 0.747 | 0.469 |
| Estimated maximum volume (log) | 1, 12 | –0.646 | –0.917 | 0.377 |
| Area of opening (square-root) | 1, 12 | –2.028 | –0.768 | 0.457 |
| Potential depth (square-root) | 1, 12 | –0.466 | –0.769 | 0.457 |