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Water-filled tree holes and their use by small vertebrates: a comparative study between spring and summer

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Abstract

Water-filled tree holes represent unique microhabitats that support forest biodiversity by providing habitats for specialized insect species and serving as a water and food source for small vertebrates and arthropods. However, studies on the use of water-filled tree holes by terrestrial organisms in temperate forests remain limited. To address this research gap, 12 tree holes at ground level (< 2 m) in an 8-hectare managed forest area near Sihlwald, Switzerland, were monitored using motion-sensor cameras and fake plasticine larvae were exposed to the water surface. Videos were analyzed to identify species, behaviors, and activity patterns, while fake larvae were used to assess predation pressure. A total of 1516 observations were recorded, and results showed that mice were the primary users of the tree holes, with significantly higher activity in spring than in summer. Additionally, mice were observed to be much more active during nocturnal hours than during daylight hours. Other small mammals, birds, amphibians, and insects were also observed but in much smaller numbers. Lastly, about half of the exposed fake plasticine larvae showed marks of predation. While there is no clear seasonal difference in predation pressure, mice and arthropods participated significantly in the proportion of attacks, with arthropods attacking significantly more during summer. This study highlights the ecological importance of water-filled tree holes in promoting biodiversity and underscores the need for further research to better understand their role in temperate forest ecosystems.

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Introduction

Water-filled tree holes (hereafter THs), also known as dendrotelmata, represent unique microhabitats formed when water accumulates in tree cavities, either due to tree architecture or decay (Kitching, 2000; Petermann and Gossner, 2022). These microhabitats serve as valuable habitats for the development of many, often specialized, organisms including various insect taxa, such as Diptera and Coleoptera, which complete part of their life cycle in these aquatic environments. These insect larvae, in turn, become a valuable food source for predatory insects and small vertebrates (Gossner, 2018). In addition to their function as feeding grounds, these places are used by vertebrates and insects for drinking and bathing, particularly by birds, amphibians and small mammals (Kirsch *et al.*, 2021). These ecological roles become especially relevant during periods of drought when water or food sources may become scarce in certain regions.

While numerous studies have focused on invertebrates developing in these aquatic microhabitats in temperate forests (Fashing, 1975; Schmidl, Sulzer and Kitching, 2008; Gossner *et al.*, 2016; Kirsch *et al.*, 2021), studies investigating the use of THs by vertebrates in these ecosystems remain limited. Recent work by Kirsch et al. (2021) and Gossner et al. (2020) represents one of the first attempts to explore vertebrate interactions with THs in temperate forests.

Building on these findings, I hypothesize that mice and squirrels will be the most frequent users of THs, primarily for foraging and drinking (Kirsch *et al.*, 2021). In contrast, birds are expected to exhibit lower activity at ground-level THs, as they typically prefer higher canopy strata to avoid predation risk (Lima and Dill, 1990). However, birds may display additional behaviors, such as bathing, especially during summer months when temperatures are higher (Kirsch *et al.*, 2021). On the other hand, mice's activity is expected to be most frequently during nocturnal hours, while birds' activity during daylight hours (Lima and Bednekoff, 1999; Viviano, Scarfò and Mori, 2022).

Furthermore, I hypothesize that seasonal differences will influence the use of THs. Specifically, mice are expected to exhibit a higher proportion of drinking observations in summer than in spring, likely due to reduced availability of alternative water sources. In contrast, birds may show consistent drinking patterns across seasons but could increase bathing behavior in summer due to higher temperatures. Feeding activity in birds, however is anticipated to peak in spring, coinciding with the breeding season (Dawson *et al.*, 2001). Lastly, predation pressure will be assessed by installing artificial larvae and examining them for predator marks. It is expected that small mammals will be the primary group responsible for the marks on the fake caterpillars (Gossner *et al.*, 2020).

This study aims to extend the research of Kirsch et al. (2021) and (Gossner *et al.*, 2020) by comparing the use of THs during spring and summer. By investigating seasonal variations in TH usage, this research will contribute to a deeper understanding of the ecological roles of these microhabitats and their importance for biodiversity conservation.

Methods

Study area and design

The study was conducted immediately outside Sihlwald, a temperate forest approximately 10 km south of Zurich. It is a natural forest reserve in the Sihl Valley and covers around 970 hectares of mostly mixed beech forest. Together with the surrounding forests, the Sihlwald forms the largest natural contiguous mixed deciduous forest in the densely populated Central Plateau (Zürich, no date). For this project, a managed forest site right outside the reserve of Sihlwald (**Fig. 1**), located in Langnau-Gattikon (ZH) and measuring about 80’000 m² (8 hectares), was selected (47.4821 N, 8.5235 E). The area is dominated by beech (*Fagus sylvatica*) and on average it “has an annual temperature of 9.8 °C and a yearly precipitation volume of 1022 mm” (*Garden of the XXI Century in Zürich*, no date). Within this area, 18 tree holes at ground level (< 2 m) had been previously identified and cataloged (Yaremchuk & Gossner, unpublished), corresponding to a density of approximately one tree hole per 4000 m². Of these, 12 ground tree holes (< 2 m) distributed across the site were chosen for the study based on size (minimum 100 cm^2) (**Fig. 1**). Additionally, tree holes were selected from different individual trees.

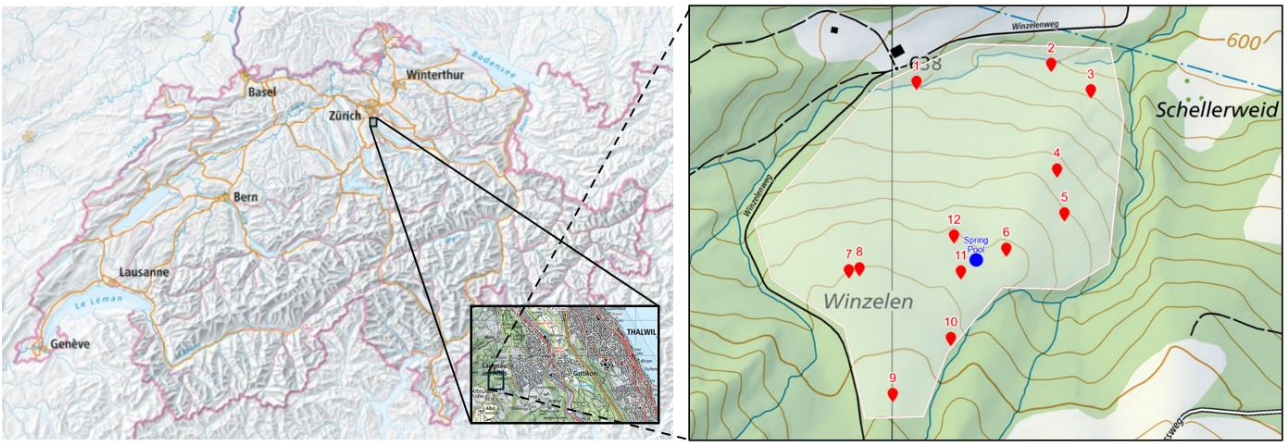


Figure 1. Map of the research area within Langnau-Gattikon (ZH). The highlighted area represents the 8-hectare area where the research was conducted. The red symbols indicate the 12 tree holes used for the study, while the blue circle marks the location of the water body that was present during spring. Map source: map.geo.admin.ch (accessed on 28.11.2024).

Tree data

Data on the trees on which the selected tree holes were located were recorded. These measurements included the coordinates and altitude above sea level of each tree to determine their spatial distribution within the study area. The tree diameter at breast height (DBH) was measured as an indicator of tree size. The openness of the canopy surrounding each tree was assessed, as it could influence microclimatic conditions such as light availability and moisture levels. Additionally, the distance from water bodies (streams, spring pool; **Fig. 1**) was measured in both spring and summer to consider the proximity of each tree to potential additional sources of water.

Tree holes data

Detailed measurements of the tree holes were collected to characterize their physical features and potential ecological function. These included the length, width, and total depth of each tree hole to describe its overall dimensions. The depth covered by water at the time of measurement (start of each study period) was recorded to assess its water-holding capacity and potential as a habitat for aquatic organisms (Petermann and Gossner, 2022). From these measures two estimates of water volume were calculated: the total volume of water that each tree hole could theoretically contain, and the volume of water present at the start of the experiment. This was done by approximating the TH’s morphology to either a cone or a triangular pyramid and then calculating its volume using TH length, width and height (or water height for the initial water volume) based on the appropriate formula. The lateral height and lateral width of the tree hole openings were measured to determine the size of the entry point, along with the estimated opening area, both of which could influence access and visibility to organisms. The height of each tree hole above the ground and the direction it faced were noted to evaluate potential effects of accessibility and sun or wind exposure.

Visitation rates of tree holes by terrestrial organisms

To monitor activities of terrestrial organisms around these tree holes, two types of cameras were deployed at a height of about 60 cm and positioned about 70 cm away from the tree holes: WINGSCAPE TimelapseCam Pro cameras to capture invertebrates and Bushnell Trophy Cam HD Essential E3 Trail cameras to capture vertebrates. Six randomly selected tree holes were monitored using WINGSCAPE cameras, which were programmed to record a 30-second video every 5 minutes. The remaining six tree holes were recorded using Bushnell cameras, which were triggered by motion sensors to capture 10-second videos upon activation, followed by a 10-second downtime. After 15 days, the cameras of the two types were swapped between tree holes, and the same recording procedure was repeated. Battery levels were checked every three days to ensure continuous operation.

Two periods of 30 days were recorded. The first one in spring between April 15, 2024 and May 15, 2024, and the second one in summer, starting on July 16 and ending on August 15, 2024.

To exclude the effect of different water levels inside the tree holes during the study period, rainwater was collected on April 27, 2024, at the WSL Institute in Birmensdorf. The collected water was stored and later used during spring and summer to fill up the tree holes during each visit (every three days).

Due to time restriction, only the Bushnell cameras with motion sensors were analyzed as part of the Master’s thesis. Each available recording from the cameras was reviewed, and the following details were documented: the species of the observed animal, the date and time of the recording, and the observed behavior. Behaviors were categorized as drinking, eating, unknown but potentially related to the tree holes, or unrelated (e.g., simply walking past the tree hole without interacting with it).

Predation pressure

Predation pressure was measured during the same two 2-week periods as the camera observations in spring and summer. Five plasticine caterpillars (length: 2 cm, width: 0.5 cm) were placed on the water surface and fixed to the trunk in each tree hole with a thread and a pin. In total, four two-week periods were assessed.

Each plasticine caterpillar was examined under a Wild Heerbrugg stereomicroscope and any marks found were analyzed and the animal group that caused the mark identified, using guides for mark identification (Low *et al.*, 2014; Michael Jeske, 2019) and checked by MMG. Additionally, for attacked caterpillars, it was recorded whether they showed a single mark or signs of heavy attack.

Food availability

To estimate food availability within each tree hole, 50 ml samples were collected at the end of each two-week period (two in spring, two in summer) using a 100 ml syringe and a tube attached if necessary. Samples were transferred to 50 ml Falcon tubes for storage. Each of the 48 samples was subsequently filtered through 125 µm and 250 µm sieves, and the retained content was examined under a Wild Heerbrugg stereomicroscope. Arthropods were identified and categorized into morphospecies using a common identification key for aquatic insects and other invertebrates (Sundermann *et al.*, 2007).

Statistical analyses

All data analyses were performed using R 4.1.2. (R Core Team, 2021). Graphs illustrating animals' activity patterns over a 24-hour period were created using the ggplot2 package (Wickham, 2011)

To test for differences in predation rates between the two seasons, a generalized linear mixed model (GLMM) was fitted using the glmer() function with a binomial error structure and a logit link function. Fixed effects included the number of larvae attacked by each predator type (e.g., small mammals, birds, insects, other arthropods, slugs) and tree hole characteristics (e.g., initial water volume, maximum water volume, height from the ground, opening size and distance from the nearest stream), and the response variable was the proportion of attacked fake larvae as cbind (number of larvae attacked vs. number of larvae not attacked). Tree hole number was added as a random factor to the model.

In addition to the overall analysis, separate GLMMs were fitted for each predator type (e.g., small mammals, birds, insects, other arthropods, or slugs) to evaluate seasonal differences in predation rates. For each model, fixed effects included season and tree hole characteristics (e.g., initial water volume, maximum water volume, height from the ground, opening size, and distance from the nearest stream), and the response variable was specified as cbind(number of larvae attacked by that predator type, number of larvae not attacked by that predator type). Tree hole number was again included as a random effect.
To evaluate seasonal differences in predation rates, estimated marginal means (EMMeans) were calculated for each model using the emmeans() function from the emmeans package (Lenth, 2016). The EMMeans represented the predicted probabilities of predation for each season, holding all other fixed effects constant. These predictions were back-transformed from the logit scale to the response scale (proportion of larvae attacked), and 95% confidence intervals were calculated to assess the precision of the estimates.

Lastly, to test for differences in predation pressure on fake larvae by mice between the two seasons, a generalized linear mixed model (GLMM) was fitted using the glmer() function with a binomial error structure and a logit link function. Fixed effects included the number of larvae of each arthropod morphospecies from the 50 ml samples, and the response variable was the proportion of attacked fake larvae by mice as cbind(number of larvae attacked by mice, number of larvae not attacked by mice), with tree hole number included as random effect.

Results

Trees and tree holes data

Table 1 provides detailed information about trees' characteristics, and **Table 2** about tree holes' characteristics.

Table 1. Summary of trees' characteristics.

Tree ID	N	E	Altitude (m. a. s. l.)	Camera height (cm)	Openness (%)	DBM (cm)	Distance from closest stream (m)
1	47.28330	8.52284	630	34	15	60	6
2	47.28344	8.52455	613	77	20	57	10
3	47.28321	8.52505	620	59	5	73	33
4	47.28253	8.52461	656	45	10	54	72
5	47.28215	8.52469	663	49	40	59	58
6	47.28185	8.52395	681	58	30	73	34
7	47.28168	8.52195	691	59	35	52	59
8	47.28170	8.52208	691	52	30	63	68
9	47.28061	8.52248	709	60	15	80	36
10	47.28109	8.52323	693	58	20	59	8
11	47.28166	8.52337	687	64	15	73	39
12	47.28197	8.52329	682	57	20	67	68

Table 2. Summary of tree holes' characteristics.

Tree Hole ID	Length (cm)	Width (cm)	Depth (cm)	Water depth (cm)	Max. water volume (cm^3)	Initial water volume (cm^3)	Height from ground (cm)	Direction	Lateral height (cm)	Lateral width (cm)	Lateral size (cm^2)
1	23	14	39	36	6279	5796	37	170 S	55	20	242
2	22	14	13	7	2002	1078	49	160 S	24	19	231
3	29	13	23	16	4336	3016	17	120 SE	26	19	283
4	15	14	14	10	1470	1050	17	45 NE	19	12	158
5	13	5	15	9	488	293	10	340 N	20	6	49
6	11	3	14	6	116	50	30	240 SO	20	6	90
7	18	6	7	5	189	135	19	90 E	19	8	114
8	25	3	13	9	244	169	13	191 S	40	6	180
9	37	9	24	13	3996	2165	11	316 NO	38	10	285
10	44	18	9	9	3564	3564	18	110 E	29	11	239
11	19	9	20	14	1710	1197	3	85 E	11	8	66
12	26	5	21	17	683	553	6	225 SO	27	4	81

Recordings

I had a total amount of 1516 observations (**Table 3**), of which 1228 occurred in spring and 288 during summer. **Table 4** provides a detailed breakdown of the number of observations per tree hole during both seasons. The observations comprised at least 14 different species belonging to four classes: mammals, birds, amphibians and insects. Only six species, though, were observed interacting with the THs: mouse, shrew, marten, robin, wren and bumblebee. In most cases the exact behavior could not be identified.

Table 3. Summary of the number of observations of each animal species per season and related behavior (FOR = foraging, DRI = drinking, UNK = unknown). Behaviors unrelated to THs (UNR) represent the combined number of observations from both spring and summer.

Class		Spring			Summer			UNR	Total
Species	Scientific name	FOR	DRI	UNK	FOR	DRI	UNK		
Mammals									
Mouse	*Apodemus* sp.	77	88	270	21	13	83	852	1399
Shrew	*Sorex* sp.	0	0	3	1	0	45	8	57
Marten	*Martes martes*	0	0	0	0	0	2	1	3
Squirrel	*Sciurus vulgaris*	0	0	0	0	0	0	1	1
Birds									
European Robin	*Erithacus rubecula*	0	0	0	0	2	2	14	18
Eurasian Wren	*Troglodytes troglodytes*	0	0	1	0	0	1	9	11
Great Tit	*Parus major*	0	0	0	0	0	0	7	7
European Blackbird	*Turdus merula*	0	0	0	0	0	0	4	4
Treecreeper	*Certhia* sp.	0	0	0	0	0	0	3	3
Eurasian Nuthatch	*Sitta europaea*	0	0	0	0	0	0	1	1
European Greenfinch	*Chloris chloris*	0	0	0	0	0	0	1	1
Song Thrush	*Turdus philomelos*	0	0	0	0	0	0	1	1
Unidentified		0	0	0	0	0	0	1	1
Amphibians									
Fire Salamander	*Salamandra salamandra*	0	0	0	0	0	0	1	1
Insects									
Bumblebee	*Bombus* sp.	0	0	6	0	0	0	2	8
All observed taxa		77	83	280	22	15	133	906	1516

Table 4. Number of observations per tree hole during spring and summer seasons.

Tree ID	Season	
	Spring	Summer
1	2	24
2	55	9
3	147	81
4	276	23
5	241	30
6	48	0
7	2	11
8	33	3
9	32	23
10	236	69
11	1	15
12	155	0

Including both related and unrelated behaviors, mammals were generally detected to be more active during nocturnal than daylight hours (**Fig. 2A**). During the night, they showed higher activity at THs in spring than in summer, while during the day (mostly afternoon), they showed higher activity in summer than spring. Birds were most active during the day, and this was consistent in spring and summer (**Fig. 2B**).

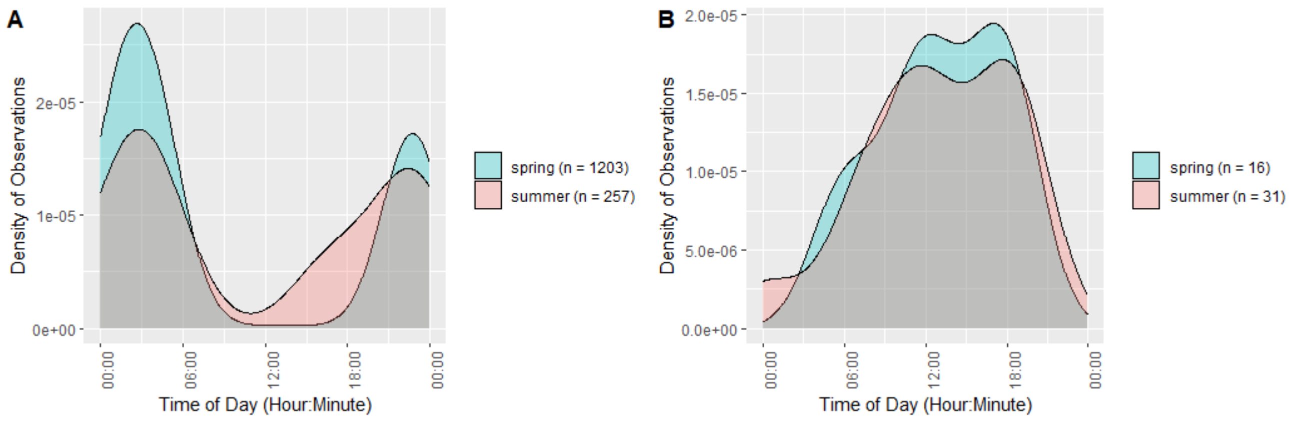


Figure 2. Activity of mammals (**A**) and birds (**B**) during the 24h of the day.

Mice were more active during the night, while they were observed much less during the day (**Fig. 3A**). This trend seems to be slightly more pronounced during spring. Shrews follow a similar trend in spring (**Fig. 3B**) but showed high activity during the afternoon in summer. Martens were only observed during evening hours, and only in summer (**Fig. 3C**). Robins were observed to be most active during the early hours of the day, with a moderate activity during the afternoon and no activity during the night in spring (**Fig. 4A**). In summer, this trend was observed to be similar but shifted to about six hours later: the activity peak was around noon, a moderate activity occurred during the evening and no activity during the night. Wrens were observed around noon and afternoon during both spring and summer (**Fig. 4B**). This trend seems to be more pronounced during spring. Great tits were observed during the morning and noon in spring, and during the (late) afternoon in summer (**Fig. 4C**). Blackbirds' observations occurred only during the morning in summer (**Fig. 4D**), while treecreepers' observations occurred during the afternoon, mainly the late afternoon (**Fig. 4E**). Bumblebees exhibited a similar activity pattern to treecreepers, with most activity occurring in the afternoon and peaking in the late afternoon (**Fig. 5**).

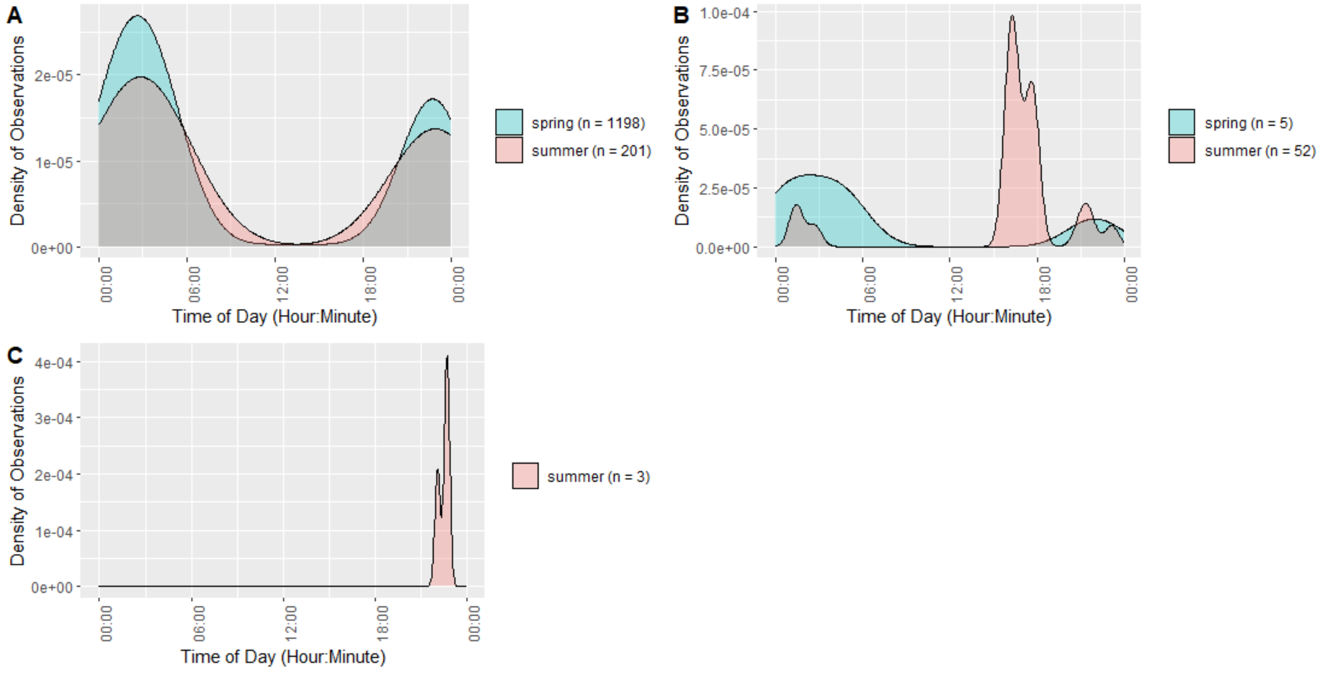


Figure 3. Activity of mouse (**A**), shrew (**B**) and marten (**C**) during the 24h of the day.

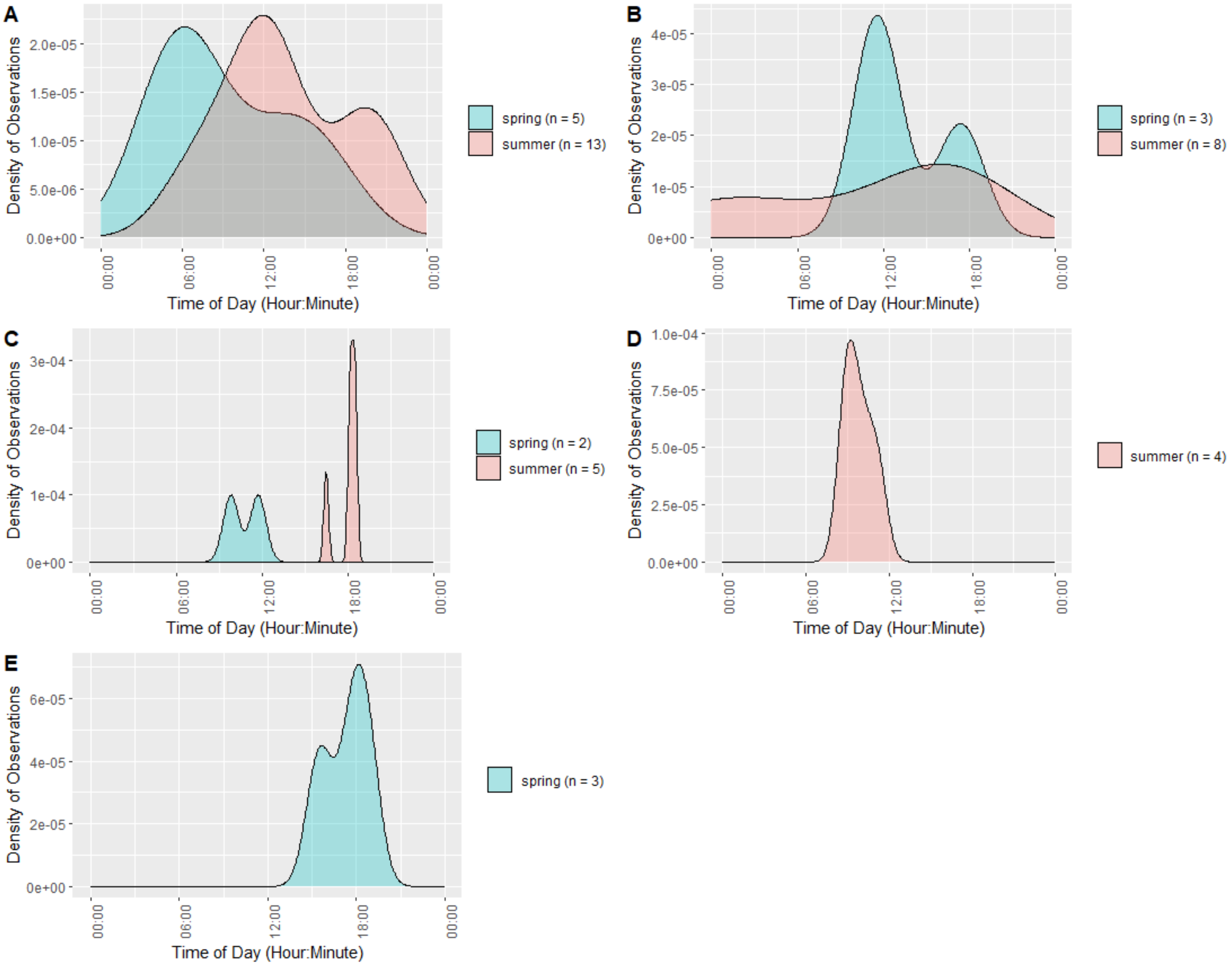


Figure 4. Activity of robin (**A**), wren (**B**), great tit (**C**), blackbird (**D**) and treecreeper (**E**) during the 24h of the day.

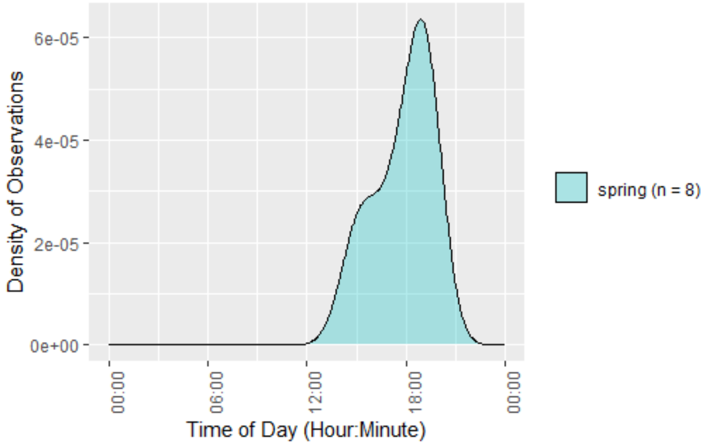


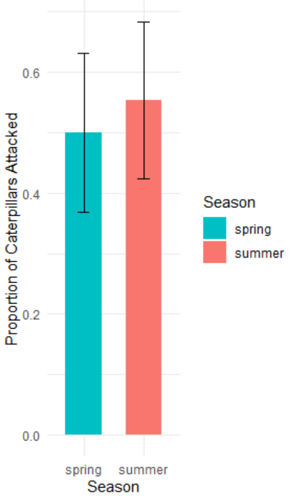
Figure 5. Activity of bumblebee during the 24h of the day.

Predation pressure

Of the 240 deployed fake larvae, 238 were recovered after the respective 2-week exposure period. In spring, 60 out of the 120 recovered larvae (50%) showed evidence of predation, while in summer, 65 of the 118 recovered larvae (55%) were attacked (**Fig. 6**). **Table 5** provides a detailed breakdown of the identified predator types and their contributions to the observed attack patterns and **Fig. 7** a graphical visualization of it.

Table 5. Number of fake larvae attacked by each type of predator in spring and summer.

Predator type	Spring	Summer
Mammals	45	35
Birds	2	0
Insects	5	0
Other Arthropods	10	33
Slugs	2	8



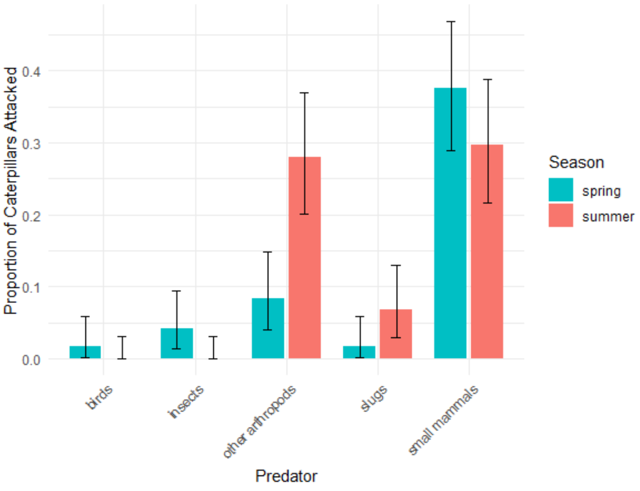


Figure 6. Proportion of fake caterpillars that were attacked each season.

Figure 7. Proportion of fake caterpillars that were attacked each season by each predator type.

The generalized linear mixed model (GLMM) revealed that predation rates of fake larvae were significantly influenced by certain predator types, while tree hole characteristics and season had no significant effects. Specifically, small mammals had a highly significant positive effect on predation rates (Estimate = 0.447, SE = 0.110, $p < 0.001$), indicating that they were major contributors to larvae predation. The group "other arthropods" also had a significant positive effect (Estimate = 0.398, SE = 0.134, p = 0.002). Birds (Estimate = 1.465, SE = 0.851, p = 0.085), insects (Estimate = 0.593, SE = 0.365, p = 0.105) and slugs (Estimate = 0.249, SE = 0.263, p = 0.344) showed positive non-significant effects. Tree hole characteristics, including initial water volume (p = 0.329), total maximum water volume (p = 0.448), height from the ground (p = 0.922), opening size (p = 0.456), and distance from the nearest stream (p = 0.270), had no significant influence on predation rates. Similarly, there was no significant seasonal difference in predation rates between spring and summer (Estimate = 0.396, SE = 0.461, p = 0.390). The random effect of tree ID had a variance of zero, suggesting that variability among tree holes did not significantly contribute to the overall variation in predation rates.

To further explore seasonal differences in predation rates, estimated marginal means (EMMeans) were calculated for each season based on the fitted GLMM. The predicted probabilities of predation were 0.643 (95% CI: 0.449–0.799) for spring and 0.728 (95% CI: 0.504–0.876) for summer. While predation rates were slightly higher in summer compared to spring, the confidence intervals overlapped, indicating that the seasonal difference was not statistically significant. These results were averaged over the levels of bird activity and back-transformed from the logit scale for interpretability.

To evaluate whether tree hole characteristics influenced predation pressure by small mammals, a generalized linear mixed model (GLMM) was fitted using the proportion of larvae attacked by small mammals as the response variable. Fixed effects included season, initial maximum water volume, total maximum water volume, height from the ground, opening size, and distance from the nearest stream. Tree ID was included as a random effect to account for variability among individual tree holes.
The model revealed no significant effects of any tree hole characteristics or season on predation pressure by small mammals. The effect of season was not significant (Estimate = -0.4173, SE = 0.2966, p = 0.159), indicating no difference in predation pressure between spring and summer. Similarly, initial maximum water volume (Estimate = -0.0008, SE = 0.0006, p = 0.208), total maximum water volume (Estimate = 0.0003, SE = 0.0007, p = 0.649), height from the ground (Estimate = -0.0510, SE = 0.0306, p = 0.101), opening size (Estimate = 0.0200, SE = 0.0078, p = 0.794), and distance from the nearest stream (Estimate = -0.0331, SE = 0.0200, p = 0.101) showed no significant influence on predation pressure.
The estimated marginal means (EMMeans) analysis for season revealed a predicted probability of predation by small mammals of 0.351 (95% CI: 0.237–0.486) in spring and 0.263 (95% CI: 0.166–0.389) in summer. Although the probability was slightly lower in summer, the confidence intervals overlapped, further indicating no statistically significant seasonal difference in predation pressure by small mammals.

Unfortunately, the GLMM for predation pressure of birds failed to converge due to numerical instability, likely caused by the combination of predictors with very different scales and potential collinearity. Efforts to rescale predictors, simplify the model, and explore alternative modeling approaches did not resolve the issue. Therefore, I was unable to reliably assess the effects of the predictor variables on bird predation pressure using this method.

The same approach was used to examine whether tree hole characteristics influenced predation pressure by insects. The analysis revealed no significant effects of tree hole characteristics or season on predation pressure. The effect of season was not significant (Estimate = -3.223, SE = 3.847e+06, p = 1.00), suggesting no difference in predation pressure between spring and summer. Similarly, initial maximum water volume (Estimate = -2.204, SE = 2.897, p = 0.447), total maximum water volume (Estimate = 1.168, SE = 3.624, p = 0.747), height from the ground (Estimate = 0.380, SE = 1.064, p = 0.721), opening size (Estimate = -1.883, SE = 1.493, p = 0.900), and distance from the nearest stream (Estimate = -1.129, SE = 1.547, p = 0.465) were not significant predictors of predation pressure by insects.
The estimated marginal means (EMMeans) analysis for season showed a predicted probability of predation by insects of 0.0205 (95% CI: 0.00475–0.0844) in spring and 0.0000 (95% CI: 0–1) in summer. Although the predicted probability was slightly higher in spring than in summer, the wide and overlapping confidence intervals indicated no statistically significant seasonal difference in predation pressure by insects.

The analysis for predation pressure by other arthropods revealed significant effects of some tree hole characteristics and seasonality. Specifically, season had a significant positive effect (Estimate = 1.553, SE = 0.404, p < 0.001), indicating that predation pressure was higher in summer compared to spring. Additionally, total maximum water volume had a significant negative effect (Estimate = -1.834, SE = 0.828, p = 0.027), meaning that higher water volumes reduced predation pressure. Opening size also showed a very significant positive effect (Estimate = 0.016, SE = 0.0047, p < 0.001), suggesting that larger openings increased predation pressure by other arthropods. Conversely, initial maximum water volume (Estimate = -0.155, SE = 0.808, p = 0.847), height from the ground (Estimate = -0.664, SE = 0.363, p = 0.067), and distance from the nearest stream (Estimate = -0.716, SE = 0.445, p = 0.107) were not significant predictors of predation pressure.
The estimated marginal means (EMMeans) analysis predicted a probability of predation by other arthropods of 0.0622 (95% CI: 0.031–0.121) in spring and 0.2386 (95% CI: 0.162–0.337) in summer. This confirms that predation pressure is significantly higher in summer, consistent with the results of the fixed-effects analysis.

Lastly, the analysis for predation pressure by slugs revealed some effects of tree hole characteristics and seasonality. Season had a marginally non-significant positive effect (Estimate = 1.541, SE = 0.816, p = 0.059), suggesting a tendency for higher predation pressure in summer compared to spring. Total maximum water volume had a significant negative effect (Estimate = -3.913, SE = 1.765, p = 0.027), indicating that higher water volumes reduced predation pressure by slugs. Conversely, initial maximum water volume (Estimate = 2.881, SE = 2.105, p = 0.171), height from the ground (Estimate = -1.742, SE = 1.039, p = 0.094), opening size (Estimate = 1.242, SE = 0.664, p = 0.062), and distance from the nearest stream (Estimate = -0.0026, SE = 0.938, p = 0.998) were not significant predictors of predation pressure by slugs. The estimated marginal means (EMMeans) analysis predicted a probability of predation by slugs of 0.0072 (95% CI: 0.0011–0.0436) in spring and 0.0326 (95% CI: 0.0089–0.1128) in summer. While the predicted probability was higher in summer than in spring, the overlapping confidence intervals suggest that the seasonal difference in predation pressure by slugs is not statistically significant, consistent with the marginal results of the fixed-effects analysis.

Food availability

The analysis of the 50 ml water samples yielded the results presented in **Table 6**. This table provides a detailed breakdown of the number of identified arthropod morphospecies found in each tree hole during the spring and summer seasons.

Table 6. Counts of larvae of arthropod larvae from tree holes, categorized by morphospecies and season (spring and summer).

Season	Tree Hole ID	Morphospecies					
		Syrphidae	Psychodidae	Scirtidae (*Prionocyphon serricornis*)	Nematocera (Culicidae)	Chironomidae (*Metriocnemus cavicola*)	Ceratopogonidae (*Dasyhelea* sp.)
Spring	1		1			1	
	2					3	
	3					22	
	4			1		2	
	5					8	
	6						
	7						
	8						
	9						
	10	10	1	1			
	11				8	4	
	12						
Summer	1					7	
	2						
	3					11	
	4			7		15	
	5					14	
	6	4				19	3
	7			1		9	
	8					16	
	9			6		4	
	10	5					36
	11						
	12					5	2

The generalized linear mixed model (GLMM) revealed that predation rates of fake larvae by mice was significantly positively correlated with the number of Syrphidae larvae (Estimate = 0.378, SE = 0.172, z = 2.197, p = 0.028), indicating that higher number of Syrphidae larvae were associated with increased odd of mice attacks on fake larvae. Other predictors, such as larvae counts of Psychodidae (Estimate = -2.536, SE = 1.735, z = -1.462, p = 0.144), Scirtidae (*Prionocyphn serricornis*) (Estimate = 0.017, SE = 0.111, z = 0.151, p = 0.880), Nematocera (Culicidae) (Estimate = 0.069, SE = 0.117, z = 0.593, p = 0.553), Chironomidae (*Metriocnemus cavicola*) (Estimate = 0.016, SE = 0.033, z = 0.476, p = 0.634), and Ceratopogonidae (*Dasyhelea* sp.) (Estimate = -0.048, SE = 0.036, z = -1.316, p = 0.188), were not significant.

Discussion

The study demonstrates that mice were the most frequent users of THs, with activity peaking during nocturnal hours. Their observed behaviors (when discernible from the videos) primarily involved foraging or drinking. In contrast, squirrels were observed on only one occasion, which was much less frequent than hypothesized. These findings align with those from Kirsch et al. (2021) regarding mice, but not for the squirrels. This discrepancy may be attributed to differences in the types of THs monitored. While this study focused exclusively on ground-level THs, Kirsch et al. (2021) included THs located closer to the canopy, which might better account for squirrel activity. Birds were observed much less frequently than mammals, which is consistent with the theory proposed by Lima and Dill (1990) that birds are generally more active in higher canopy strata to reduce predation risk. In addition, contrary to the hypothesis, birds did not exhibit bathing behavior in either spring or summer, which contrasts with findings from Kirsch et al. (2021), where bathing was observed. A reason for this may be the low sample size (number of THs monitored) and the limited duration of the study, which could have reduced the likelihood of capturing this behavior. Future studies could address this by incorporating canopy-level THs, adopting a similar approach to Kirsch et al. (2021), to determine whether this behavior is more frequent in higher strata. Regarding daily activity patterns, mice were predominantly active during nocturnal hours, while birds were active during daylight hours, further supporting the findings of Lima and Dill (1990) and Viviano et al. (2022). These results reinforce the concept that birds may avoid ground-level activity to reduce predation risk. The hypothesis that mice would show a higher activity of drinking observations in summer compared to spring was not supported by the results. In contrast, birds were observed drinking on only two occasions in summer and never in spring, offering limited evidence for seasonal differences. Furthermore, no foraging behavior was observed in birds, preventing any conclusions about their seasonal feeding activity. However, the results from predation pressure on fake larvae may (even if weakly) provide evidence of birds engaging in food-searching activity.
Lastly, the number of Syrphidae larvae was found to be significantly positively correlated with the proportion of fake caterpillars attacked by mice. This finding may suggest that mice could recognize the presence of Syrphidae larvae, or other similarly large larvae, in a specific tree hole and be more likely to visit these sites with the intent of foraging.

Although this study provides valuable insights, several factors may have influenced its scope and should be addressed in future research. An important consideration is the discrimination of behaviors, particularly distinguishing between drinking and foraging activities in mice. In some cases, the behaviors were not perfectly visible, making interpretation challenging. Generally, foraging behavior was identified by the use of the mice’s hands and a posture that appeared slightly upright, whereas drinking behavior was characterized by a steadier position with minimal head and hands movement. However, subtle variations or unclear visibility in the recordings may have impacted the accuracy of this classification. The angle of the camera also posed limitations. While the positioning appeared adequate during setup, the camera was often placed in front of the tree hole and frequently recorded animals from behind, which made it difficult to clearly observe specific actions. In a few cases, suboptimal conditions near the tree hole, such as the presence of roots, further restricted the ability to achieve ideal camera angles. These challenges may have affected the quality of the observations. Additionally, the distance of the camera from the tree hole may have influenced its effectiveness in detecting activity. Cameras placed further away captured a broader area, allowing for more observations to be made, but often included unrelated behaviors. Conversely, if the animal using the tree hole was small or moved too slowly, the motion sensor may not have been triggered, resulting in missed recordings. Finally, some cameras failed to function properly, and recordings were unexpectedly missing. This was surprising given that batteries and functionality were checked regularly, approximately every three days. These technical issues, though limited, may have contributed to the gaps in the data. Overall, these factors highlight important methodological limitations that should be addressed in future studies to improve the reliability and accuracy of behavioral observations.

Methodological improvements for future research include extending the study duration, monitoring a larger number of THs, and expanding to additional study areas. Observing behaviors across other seasons, beyond spring and summer, would also provide a more comprehensive understanding of seasonal variations in TH use. These findings contribute to understanding the ecological importance of THs as microhabitats by demonstrating that they are actively used by multiple animal species in temperate forests, spanning different levels of the food chain. Particularly notable is their role in supporting mice, providing access to food and water, which in turn serve as prey for larger mammals. This highlights the critical role of THs in maintaining forest biodiversity and ecological interactions, suggesting that forest management practices should prioritize retaining trees with tree holes.

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