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ORIGINAL ARTICLE

A three year study of the phenology of insect larvae (Coleoptera, Diptera) in water-filled tree holes in the canopy of a beech tree

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Abstract. Water-filled tree holes are abundant microhabitats in forests worldwide and are inhabited by specialized communities of invertebrates. Despite their importance, the temporal dynamics of communities within and between years are largely unknown. Here, I present a case study on the phenology of insect larvae in two holes in a beech tree (lower and upper canopy) located in southern Germany over a period of three years. I asked whether water temperature and the characteristics of insect larvae at the community and population levels are similar in periodicity every year and whether they differ in the lower and upper canopy. The water temperature in tree holes differed greatly from air temperature, and this effect was more pronounced in the lower than in the upper canopy, which resulted in a lower probability of drying out occurring in the lower canopy. This was associated with a higher species richness in the lower canopy and greater abundance of drought tolerant species in the upper canopy. There was a significant periodicity in larval abundance, biomass, species richness and body size distribution of abundant species in both tree holes, but it was not seasonal. This result indicates that unpredictable drying out of tree holes are more important drivers of tree hole community dynamics than changes in water temperature. The community of larvae in the tree hole in the upper canopy lagged behind that in the lower canopy, which indicates that most species mainly colonize the more stable microhabitats in the lower canopy. Hopefully this case study will encourage future larger-scale phenological studies to test (1) whether the patterns observed in this study can be generalized over larger spatial scales and (2) the relative importance of abiotic and biotic drivers of the dynamics of communities in tree holes.

INTRODUCTION

Ephemeral water bodies associated with living plants occur worldwide and are inhabited by a high diversity of organisms, from bacteria and protists to invertebrates and vertebrates (Kitching, 2000). Among these microhabitats, the exceptionally diverse tropical phytotelmata in green plant parts, such as leaf axils of tank bromeliads, are the most well-known (Srivastava et al., 2004, 2008; Yanoviak et al., 2006; Petermann et al., 2015). However, water-filled tree holes, called dendrotelmata, also provide microhabitats for numerous species (Kitching, 1971; Yanoviak, 2001; Schmidl et al., 2008; Gossner et al., 2016). They occur ubiquitously in temperate and tropical forests and may therefore, together with phytotelmata, represent the most abundant freshwater aquatic ecosystem worldwide (Carpenter, 1982).

In temperate forests, water-filled tree holes, comprising rot-holes and pans, occur with varying frequency. In rot-holes, which form in holes in stems, branch breaks and stumps, water has contact with the wood. In pans, which are created by stem and branch forks as well as between buttress roots, the hole is lined with bark (Kitching, 1971). Both types of water-filled tree holes occur in a many broad-leaved and coniferous species (Rohnert, 1951; Schmidl et al., 2008; Rotheray, 2013; Gossner et al., 2016), but the frequency of such structures varies between different species of trees. In Central Europe, European beech (*Fagus sylvatica* L.) is the tree species in which such water-filled tree holes occur most frequently, most likely because of its root and branch architecture and its high abundance (Rohnert, 1951). Water-filled tree holes occur between buttress roots up to a height of over 30 m in the canopy, where they can be found in stem and branch forks and holes formed when a branch breaks (Gossner et al., 2016). They fill with rain water, either directly or by stem flow. Through this process, nutrients are transferred from foliage and bark into the tree holes, and thus the chemical content of the water depends not only on the species of tree, but also on dry atmospheric depositions on the bark (Carlisle et al., 1966; Eaton et al., 1973; Paradise & Dunson, 1998). The main source of energy for the saprophagous communities in these tree holes is leaf litter, which also varies in quality between different species of trees (Fish & Carpenter, 1982; Carpenter, 1983), supplemented mainly by dead arthropods (Yee & Juliano, 2006). The varying nutritional conditions among tree holes affect the reproduction of individual species of invertebrates (Mercer, 1993), as well as the composition of invertebrates inhabiting holes in particular species of trees (Rohnert, 1951).

Early studies mainly describe the ecology of water-filled tree holes and the species that inhabit them (Varga, 1928; Theinemann, 1934; Rohnert, 1951; Kitching, 1971). Most later studies in Central Europe have involved sampling tree holes at only one point in time and have addressed topics such as the preference of insect species for a particular water chemistry (Schmidl et al., 2008), microhabitat (Rotheray, 2013) or forest management (Gossner et al., 2016; Petermann et al., 2016). Studies on the temporal dynamics of species inhabiting water-filled tree holes have mostly focused on selected species or species groups over one or more annual cycles, e.g. a few species of mosquito (Sinsko & Craig, 1979; Bradshaw & Holzapfel, 1984), Syrphidae (Rotheray, 2013; Rotheray et al., 2016), a Ceratopogonid (Kitching, 1972b), or a Chironomid (Kitching, 1972a), or are anecdotal (von Brandt, 1934; Rohnert, 1951). Studies on the phenology of whole communities in water-filled tree holes are rare and mostly restricted to subtropical rain forests (Kitching, 1983, 1987; Kitching & Beaver, 1990) or to artificial tree holes (Ptatscheck & Traunspurger, 2015). The limited number of quantitative studies focusing on temporal population dynamics of whole insect communities in water-filled tree holes in temperate forests are mostly restricted to one entire season (Kitching, 1971; Woodward et al., 1988; Green, 1990; Barrera, 1996; Devetter, 2004; Paradise, 2004) or to an even shorter period (Sota, 1996). I am aware of only two studies, conducted at the same location in North America, that considered a period of more than one year (Paradise et al., 2008; Smith et al., 2009). Thus, it is unclear whether the communities in European water-filled tree holes exhibit a clear seasonal periodicity or vary in their occurrence between years. Because tree holes are highly variable in their physical and chemical characteristics (Gossner et al., 2016), it is difficult to compare different tree holes that are sampled at different times (space for time substitutions). However, sampling the same tree hole over time without considerably influencing the organisms that inhabit them is a great challenge.

Here, I studied the temperature and the communities of insects in two tree holes at different heights in the canopy of a beech tree over a period of three years by subsampling. I determined whether water temperature and the characteristics of insect larvae at the community and population levels are similar between years and whether they differ in the lower and upper canopy. With this investigation I aimed to test the suitability of taking small water samples for studying the temporal dynamics of insect larvae in tree holes and pose further questions about the temporal dynamics of insect communities developing in water-filled tree holes that should be addressed at larger spatial scales in the future.

MATERIALS AND METHODS

Study site and tree

Monitoring of temperature and occurrence of insect larvae was done over a period of three years in an old broad-leaved forest ("Schlemmerholz") near Freising, Germany. This forest is part of the "Oberbayerisches Tertiärhügelland" at an altitude of 480 m a.s.l. Under natural conditions, the region would be dominated by colline to high montane beech forest (*Fagus sylvatica* L.). Owing to management for economic reasons, however, this forest is now dominated by spruce (*Picea abies* L.), and broad-leaved forests are restricted to small patches. The mean annual precipitation is 750–800 mm and the mean annual temperature is 7–8°C.

The broad-leaved forest studied is multi-layered, 1 ha in size and dominated by European beech (*F. sylvatica*) and pedunculate oak (*Quercus robur* L.) (Fig. S1-1). In addition, there are low proportions of other broad-leaved species of trees (*Acer pseudoplatanus* L., *A. platanoides* L., *Ulmus glabra* Huds., *Fraxinus excelsior* L., *Carpinus betulus* L.) and conifers [*P. abies*, *Pinus sylvestris* L., *Pseudotsuga menziesii* (Mirbel) Franco]. Twenty four tree holes per hectare were recorded in the area of forest studied (mapped on 15 June 2017), which is an average value when compared with counts recorded in a large-scale study of tree holes in beech forests in two regions of Germany (Fig. S1-2) (Gossner et al., 2016).

The beech tree studied (48.40°N, 11.71°E) had a diameter of 75 cm and a height of 35 m (Fig. S1-1). Two water-filled holes at the bases of branches at two different heights (10.2 m, 20.2 m) were studied over a period of almost three years.

Abiotic variables

The maximum volume of the tree holes was measured by removing all the water and detritus from the holes and filling them completely with rainwater one year after the end of the study, on 15 June 2017.

Temperature in the tree holes was measured using high-resolution Thermochron® iButtons® DS1921Z-F5 in water-proof protective capsules DS9107 (Fig. S1-1). The temporal resolution of measurements was one hour. Loggers were installed on 17 October 2013 and data were retrieved on average once every two months. The last measurement was recorded on 25 May 2016 because the batteries failed during the following sampling interval.

Data on air temperature at 2 m above the ground and precipitation were taken from a nearby weather station of the Bavarian State Research Center for Agriculture (Freising BAM008, 48.41°N, 11.72°E, 470 m a.s.l., distance to the tree studied ca. 700 m). Data at 1 h intervals were obtained from the online database. The absolute difference between water temperature in the tree holes and air temperature was calculated at 1 h intervals.

Sampling and morphometric measurements of insect larvae

It is difficult to sample insect larvae to gather reliable data on temporal patterns over time without affecting the communities. A water sample of 15 ml was taken from both holes approximately once every two months. This volume is 10% of the median volume recorded in June in holes with a maximum volume of > 100 ml in the canopy of beech trees (Gossner et al., 2016). This small sample volume was used to minimize disturbance of the system. Samples were only collected when tree holes were either completely or almost completely full of water in order to avoid the error that would result from sampling a greater concentration of larvae in a smaller volume of water. Prior to sampling, the water in the tree holes was first stirred to homogenize the distribution of larvae and then sampled with a syringe (5 mm diameter opening allowed the sampling of large larvae such as those of *Myathropa florea*). Each sample was transferred to a Falcon tube and cooled before the larvae were processed in the laboratory.

An additional 15 ml sample of water was taken on 15 June 2017, followed by a complete harvesting of the tree hole contents to determine whether the earlier samples reflected the community composition of the entire tree hole. The contents of the tree holes were processed in the following ways: (1) the detritus was flushed through sieves of different mesh sizes and finally through filter paper; (2) all detritus retained by the sieves with a mesh size ≥ 0.5 mm was classed as coarse debris, and the remaining fractions as fine debris; (3) all fractions were searched for insect larvae, which were subsequently transferred to vials containing 70% ethanol and stored at 5°C until further processing. The detritus was dried in a drying oven for 24 h at 70°C and then weighed.

Insect larvae were identified to family using the following identification keys for common insect larvae (Stehr, 1987; Nilsson, 1996; Stresemann et al., 2005). For genus and species identification of Diptera, Pankratova (1970), Cranston (1982) and Lindegaard (1997) were used for Chironomidae, Nilsson (1997) for Ceratopogonidae, Utrio (1976), Cranston & Judd (1987) and Mohrig (1969) for Culicidae, Rozkošný & Gregor (2004) for Muscidae, Dixon (1960), Hartley (1961) and Rotheray (1993) for Syrphidae, Nilsson (1997) for Psychodidae and Nilsson (1997) for Tipulidae. For species identification of Coleoptera, family Scirtidae, the key of Klausnitzer (1996) was used.

The body lengths and widths, and width of the head capsules (when present) of all the larvae in the 15 ml samples were measured using a measuring eyepiece. The body length of each larva in the additional sample collected in 2017 was measured using a Leica DVM6 Digital microscope at WSL.

Biomass was calculated as dry weight based on body length and order-specific constants given in Smock (1980):

$$\ln W = \ln a + b*\ln L \qquad (1)$$

where W = dry weight, and a, b = regression constants obtained from regressions of dry weight as a function of body length.

Data analyses

All analyses were done using R version 3.2.4. Time series analyses were conducted to compare the lower and upper tree holes throughout the three years sampled in terms of (1) the mean hourly water temperature, (2) the absolute difference between tree hole water temperature and air temperature, (3) community parameters: (a) total number of individuals, (b) biomass, (c) species richness per 15 ml sample, and (4) the number of individuals and mean larval body length per 15 ml sample of the two most abundant species.

For analyzing periodicity, partial autocorrelations of each time series (lower hole, upper hole) were done separately and cross-correlations between the two series were calculated using the acf function in R (see Venables & Ripley, 2002). The partial correlation coefficients were estimated by fitting autoregressive models of successively higher orders. The lag was plotted in units of time, not in number of observations.

RESULTS

The lower tree hole (exposure SE, depth 28 cm, length 25 cm, width 14 cm, volume 2600 ml) was larger than the upper hole (exposure SW, depth 18 cm, length 30 cm, width 13 cm, volume 1300 ml). The weight of detritus was 165.85 g (coarse debris 81.94 g, fine debris 83.91 g) in the lower and 37.83 g (22.94 g, 14.90 g) in the upper hole.

Temperature and precipitation

Air temperature was between –13.7 and 34.6°C, and precipitation between 0 and 33.1 mm per hour throughout the study period. Both variables fluctuated considerably and varied between years, resulting in an acyclic drying out of tree holes (Fig. 1). The water temperature was between –2.25 and 25.38°C in the upper and between –2.25 and 24.25°C in the lower hole, and differed greatly at the two heights at specific times and seasons. The water temperature in the lower tree hole differed more strongly from air temperature than that in the upper tree hole. This finding is illustrated by the higher peaks temperatures recorded in the upper hole in Fig. 1, and supported by the results of the partial autocorrelation analyses (Table 1, Figs S3-1 to S3-3). This effect was, however, stronger in autumn, winter and spring than in summer (Fig. 2). The upper tree hole dried out six times during the three-year study, and during two of these events not even moist mud remained (see strong peaks in the red line in spring 2015 in Fig. 1; Fig. S4-2). In contrast, the lower tree hole dried out twice and moist mud still remained. Nevertheless, both tree holes froze completely in winter (Fig. 1).

Comparison of samples with the complete harvest

The harvest of the complete contents of the two holes collected in 2017 contained a total of 372 (lower tree hole) and 163 (upper tree hole) insect larvae. In the 15 ml samples taken before the complete harvest, there were 23 (6% of the complete harvest) and 19 (12%) larvae in the samples from the lower and upper tree holes, respectively. All species that occurred in the tree holes were detected in the samples (Fig. 3). In the complete harvest, the larvae of the most abundant species, *Metriocnemus cavicola* Kieffer, 1921 (also known as *M. martinii* Thienemann, 1921) were distinctly smaller in the lower than in the upper tree hole. This was also recorded in the 15 ml sample, but was less pronounced.

Yearly occurrence of the species

In total, the larvae of at least nine species (not all individuals were identified to species level) of insects were recorded in the two tree holes in this three year study (Fig. 4 & Supplement S4). More species were recorded in the lower (7) than in the upper hole (6), three occurred exclusively in the lower hole, and two exclusively in the upper hole. The most abundant species were *M. cavicola* and *Prionocyphon serricornis* (Mueller, 1821) (Fig. 4). The much higher abundance of *P. serricornis* in the lower hole and the exclusive occurrence of Culicidae [*Aedes geniculatus* (Olivier, 1791), *Anopheles* sp.] in the lower hole and of Psychodidae in the upper hole, may indicate a stratification in the occurrence of these species. The raw species data is in Supplement S5.

Shortly after the tree holes filled with water during rainfall following a dry period (Fig. S3-10) and as soon as the ice began to melt in winter, insect larvae were observed. Many live larvae of *P. serricornis* were observed in the water above the ice, indicating that the larvae can survive when the water is completely frozen. In contrast, *Myathropa florea* sometimes became frozen in the ice and died (Fig. S4-1). In addition, terrestrial insects such as honey bees and beetles used the tree holes as a source of water (Fig. S4-2), especially during dry periods when other sources of water are scarce. In contrast, slugs were

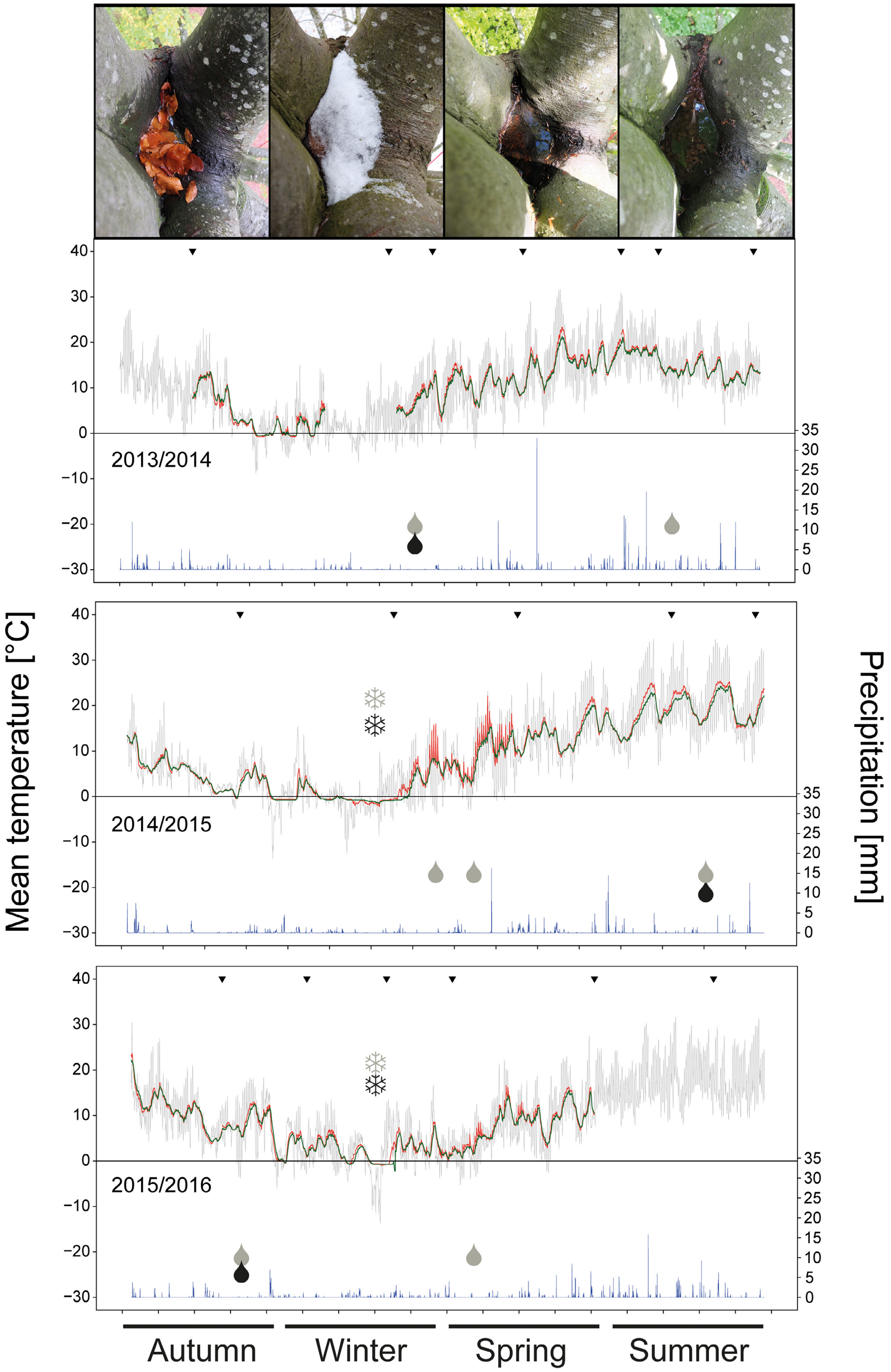

Fig. 1. Mean temperature each hour of the water in tree holes formed where branches fork 10.2 m (green line) and 20.2 m (red line) above the ground. The grey line is mean temperature and blue bars precipitation, both measured at a nearby weather station outside the forest. Black triangles indicate dates when samples of water were collected from the tree holes. Water droplet symbols indicate dates when tree holes dried out, and ice crystal symbols indicate dates when the water in the tree holes was completely frozen (black: 10.2 m, grey: 20.2 m). The photographs show the tree hole at 20.2 m above the ground in the four seasons.

Table 1. Summary of the results of the partial autocorrelation analyses of the periodicity in the lower and upper tree holes, and of the cross-correlation between the upper and lower holes. Water temperature and the difference between the water temperature and air temperature, as well as insect communities and populations (only abundant species), were considered. Analyses were done using the autocorrelation function ACF in R (see Venables & Ripley, 2002). Details of the analyses are shown in Figs S3-2 to S3-10.

	Lower hole	Upper hole	Cross-correlation lower vs. upper hole
Water temperature	Significant periodicity	Significant periodicity	– positive changes in the upper hole are associated with negative changes in lower hole and vice versa
	→ stronger periodicity in the upper hole		→ lower hole water temperature lags behind upper hole water temperature
Difference between water temperature and air temperature	Significant periodicity	Significant periodicity	Weak cross-correlation
	→ greater buffering against changes in air temperature in the lower hole		
Number of insect larvae	Significant periodicity	Significant periodicity	– for smaller time units (lags 3 and 4), positive changes in the lower hole are associated with negative changes in the upper hole and vice versa – for larger time units (lag 7), positive changes in the lower hole are associated with positive changes in the upper hole
	→ stronger periodicity in the upper hole		→ insect abundances in the upper hole lag behind those in the lower hole
Biomass of insect larvae		Significant periodicity	– for small and large time units (lags 1, 2, 3 and 6), positive changes in the lower hole are associated with positive changes in the upper hole – for intermediate time units (lag 5), positive changes in the lower hole are associated with negative changes in the upper hole and vice versa
	→ stronger periodicity in the upper hole		→ insect biomass values in the upper hole lag behind those in the lower hole
Insect larvae species richness		Significant periodicity	– for smaller time units (lags 4 and 6), positive changes in the upper hole are associated with negative changes in the lower hole and vice versa – for larger time units (lag 7), positive changes in the lower hole are associated with positive changes in the upper hole
	→ stronger periodicity in the upper hole		→ species richness in the upper hole lags behind that in the lower hole
Number of individuals of *Metriocnemus cavicola*		Significant periodicity	– positive changes in the lower hole are associated with negative changes in the upper hole and vice versa (significant at lags 3, 6 and 8)
	→ stronger periodicity in the upper hole		→ abundances in the upper hole lag behind those in the lower hole
Mean body length of *Metriocnemus cavicola*		Significant periodicity	– positive changes in the upper hole are associated with positive changes in the lower hole at lags 3, 5 and 8 – positive changes in the upper hole are associated with negative changes in the lower hole and vice versa at lags 2, 7 and 9
	→ stronger periodicity in the upper hole		→ mean body sizes in the lower hole lag behind those in the upper hole
Number of individuals of *Prionocyphon serricornis*		Significant periodicity	– for smaller time units (lag 1), positive changes in the lower hole are associated with positive changes in the upper hole – for larger time units (lag 4), positive changes in the lower hole are associated with negative changes in the upper hole and vice versa
	→ stronger periodicity in the upper hole		→ abundances in the upper hole lag behind those in the lower hole
Mean body length of *Prionocyphon serricornis*	Significant periodicity		– for smaller time units (lags 3, 5 and 6), negative changes in the lower hole are associated with negative changes in the upper hole – for larger time units (lag 8), negative changes in the lower hole are associated with positive changes in the upper hole and vice versa
	→ stronger periodicity in the lower hole		→ mean body sizes in the upper hole lag behind those in the lower hole

observed frequently in the tree holes during wet periods (Fig. S4-3).

The phenology of insect communities in tree holes varied between years and between the upper and lower canopy (Fig. 5). Surprisingly, the number of individuals in the holes at both heights peaked in winter in the first two years, but in summer in the third year. The partly synchronous and partly asynchronous temporal development of abundances in the upper and lower holes was confirmed by the partial autocorrelation analyses (Table 1, Fig. S3-4). Overall, the communities in the upper hole seemed to lag behind those in the lower hole (Table 1, Figs 5 & S3-4).

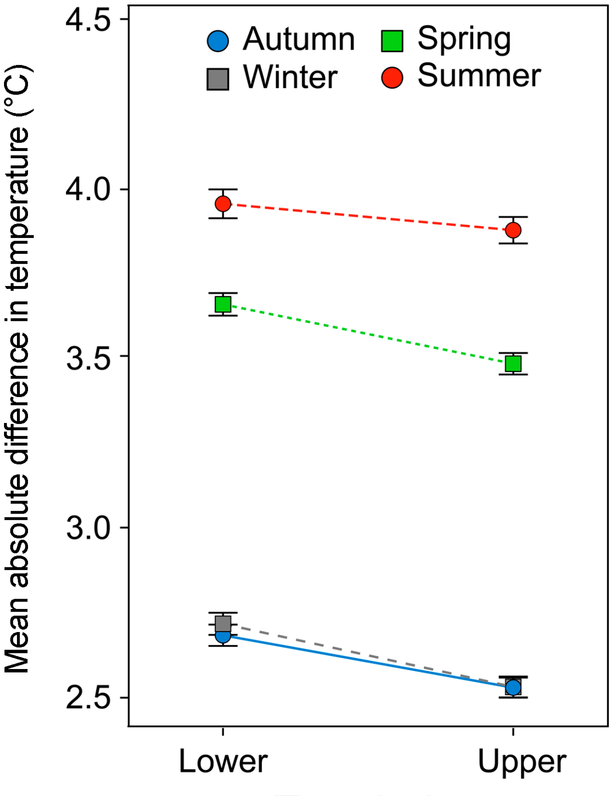

Fig. 2. Mean absolute difference (± SE) between water and air temperature across all measurements per season in the the upper and lower tree holes in autumn, winter, spring and summer. Air temperature was measured at 2 m above ground at a nearby weather station.

Insect biomass in the lower canopy also peaked in winter in the first two years, but in autumn in the third year. In contrast, insect biomass in the upper canopy was overall lower than in the lower canopy and showed only one conspicuous peak in autumn in the second year. The partly synchronous temporal development of abundance in the upper and lower holes was confirmed by the partial autocorrelation analyses (Table 1, Fig. S3-5). Overall, the communities in the upper hole seemed to lag behind those in the lower hole (Table 1, Figs 5 & S3-5).

The highest species richness in the lower canopy occurred in winter in the first, in winter/spring in the second and in winter/summer in the third year. In the upper canopy, most species were found in winter and summer in the second year. Partial autocorrelation analyses supported the partly asynchronous temporal development of species richness in the upper and lower holes (Table 1, Fig. S3-5). Overall, the communities in the upper hole seemed to lag behind those in the lower hole at some point and vice versa at other points (Table 1, Figs 5 & S3-5).

Regarding the two most abundant species, *M. cavicola* reached a maximum of 16 individuals per 15 ml in the lower tree hole and 14 in the upper hole. The maximum abundance of *P. serricornis* per sampling event was 7 individuals in the lower and 3 individuals in the upper hole. Both species showed a similar phenology in the lower and upper tree holes, but the time of maximum abundance differed among years. The evidence for periodicity in the abundance and body length of *M. cavicola* was stronger in the upper hole than in the lower hole. Interestingly, the abundance in the upper hole lagged behind that in the lower hole, while a different pattern was recorded for body size (Table 1, Figs 6, S3-6 & S3-7). The continuous occurrence of late instar larvae of *M. cavicola* over several months indicates repeated colonization events. The evidence for periodicity in the abundance of *P. serricornis* was stronger in the upper than in the lower hole, and a similar pattern was recorded for body size (Table 1, Figs 6, S3-8 & S3-9). The abundances and mean body sizes in the upper hole lagged behind those recorded in the lower hole (Table 1, Figs S3-8 & S3-9). The phenology of other species also seemed to differ among years (Fig. S2-1), but a more detailed analysis was not possible because of the low overall abundance. Interestingly, the phenology of *Dasyhelea* sp. seemed to follow an opposite pattern to that recorded for *M. cavicola*.

DISCUSSION

The present study is, to my knowledge, the first three year study of the phenology of dendrotelmata-inhabiting insects in natural tree holes in Central Europe. This study is based on data from two tree holes in one tree, and thus, the results cannot be generalized. However, some interesting patterns were recorded that should encourage future research at larger spatial scales. First, the periodicity at the community and population levels in both tree holes were not strongly linked to season, leading to different patterns in different years. It is likely that the variation in temperature and tree-hole-filling precipitation between years, leading to acyclic drying out of tree holes, determines the periodicity of communities of insect larvae in dendrotelmata. Second, the lower species richness in the upper tree hole might indicate harsher conditions high in the canopy. It is likely that tree holes in the upper canopy are less well buffered against changes in air temperature than those in the lower canopy and thus are more likely to dry out.

Abiotic temporal patterns

The volume and the pH and oxygen saturation of the water in the tree holes studied were within the range of values recorded in a large-scale study on tree holes published by Gossner et al. (2016), except for the extremely high value of oxygen saturation recorded in the upper hole (42%) (Fig. S1-2). The range in water temperature, from freezing to more than 25°C, is within the range previously reported (von Brandt, 1934; Rohnert, 1951; Schmidl et al., 2008; Gossner et al., 2016), although most of these studies did not include the winter season. That the water temperature in winter did not go below –3°C is in accordance with the findings of Rohnert (1951). Although there is a clear seasonal pattern in water temperature in the water-filled tree holes studied, which followed changes in air temperature, water temperature was mainly determined by the height above ground of tree holes and by irregular precipitation that fills the tree holes, mainly via stem flow.

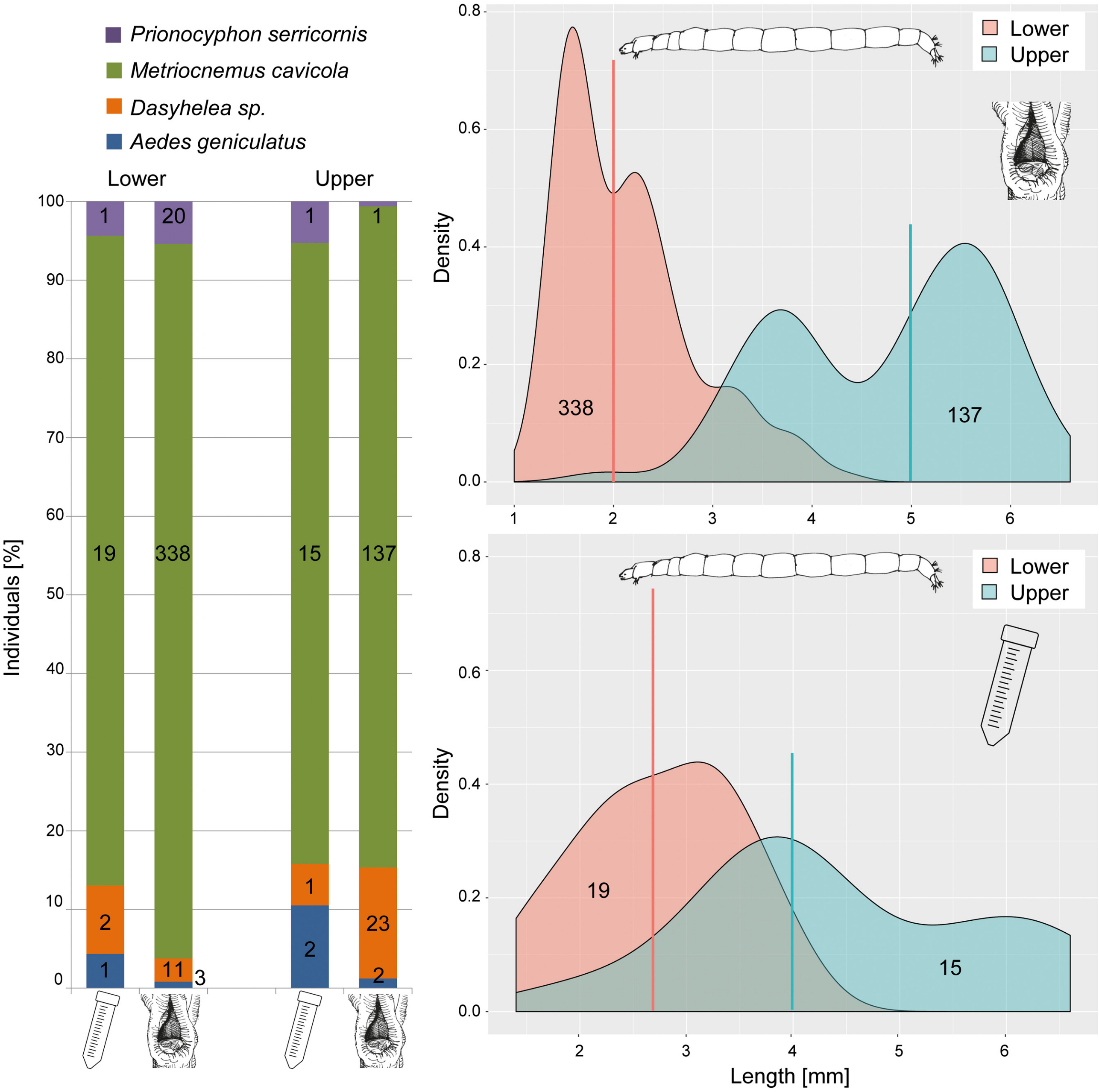

Fig. 3. Comparison of the communities in the 15 ml samples (Icon Falcon tube) and complete harvest (Icon tree hole) collected in June 2017, one year after termination of the study. Left: Community composition in the lower and upper tree holes; numbers refer to the number of individuals. Right: Size distribution of *Metriocnemus cavicola* in the complete harvest (top) and the 15 ml samples from the lower and upper holes (bottom). The vertical lines indicate the median size. The Falcon tube icon was created by Julie Ko from Noun Project.

The temperature of the water in the tree holes is buffered against air temperature (see also von Brandt, 1934; Mayer, 1938; Rohnert, 1951), but the extent of such buffering in forests most likely decreases with height above the ground and also depends on exposure. Independent of height and exposure, however, tree holes seem to dry out regularly, particularly in late winter/early spring before bud burst, and completely freeze in winter, but the frequency of these events increases with height. Freezing of water in tree holes in winter is also reported in early studies (Rohnert, 1951).

Sampling temporal dynamics of tree hole communities

To study temporal dynamics of tree hole communities, sampling was conducted bimonthly by collecting a 15 ml sample of water from each hole. This was done because (1) species identification and measurements in the field were not feasible and thus sampling of complete tree holes, as proposed by Yanoviak & Fincke (2005), was not practical; and (2) insect dynamics in tree holes vary enormously between habitats (Kitching, 2000), and thus sampling different tree holes at different points in time was not a suitable solution. It is likely that the sampling method used in the

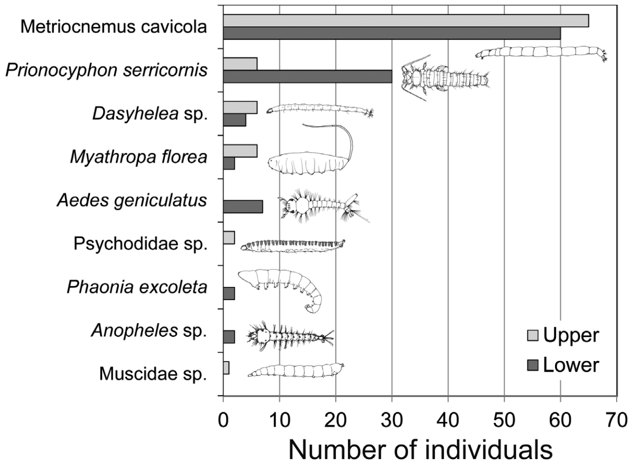

Fig. 4. Overview of the nine species that were recorded in one or other, or both, of the two tree holes studied (Lower 10.2 m and Upper 20.2 m above ground level) between 2013 and 2016.

present study is suitable and practical for analyzing temporal patterns, provided that tree holes are not too small, i.e. have a volume of at least one liter, because (1) all species in the complete harvest collected during the additional assessment in 2017 were detected in the 15 ml samples; (2) all of the more abundant species and a few of the less abundant species that were found in a previous large-scale study on insect larvae in water-filled tree holes in Central European beech forests (Gossner et al., 2016) were found in the samples collected in the present study; (3) the difference in the size distribution of the most abundant species, *M. cavicola*, between the lower and upper tree holes in the complete harvest collected during the additional assessment in 2017 was consistent with the difference observed in the 15 ml samples; and (4) results showed a clear periodicity in the communities and in the phenology of particular species.

Seasonality in the insect communities

There was no clear seasonality in the communities of larvae in tree holes, which is in line with observations in moist deciduous woodlands in Great Britain (Kitching, 1971) and in subtropical forests in Queensland, Australia (Kitching, 1983, 1987; Kitching & Beaver, 1990). Kitching (2000) proposes that the lack of seasonality in regions with pronounced seasons might be due to the slow development of saprophagous insect larvae, which is associated with the low nutritional value of their food. He assumes that seasonality occurs only in locations where holes dry out completely for long periods of time. In contrast, there was a clear periodicity in the communities studied, which was independent of season, although the tree holes studied were full of water most of the time during this study. This finding tends to indicate that the unpredictable occurrence of drying out might result in non-seasonal patterns. Culicidae and *Prionocyphon* larvae might in general have a low resistance to drought (Srivastava, 2005) and therefore exclusively occur in tree holes in the lower canopy that are less likely to dry out. Species are well adapted to these unpredictable events as their less sensitive stages, such as eggs or pupae, can survive such extreme periods. Some species of the families Chironomidae and Ceratopogonidae, as well as *M. florea* and *P. serricornis*, can survive as larvae, even when the water in a tree hole is completely frozen, as observed in the present study (see also von Brandt, 1934; Rohnert, 1951). Larvae of Scirtidae often overwinter in water above the ice layer and pupate in spring (Barrera, 1996; own observations). Some species might, however, be more sensitive in particular stages; for example, late larval stages of *M. florea* did not survive freezing for four weeks in the upper tree hole in one year. This is also reported by Rotheray (2013) for different species of Syrphidae. Although Rohnert (1951) proposes that larvae might be able to survive freezing conditions lasting 3–4 weeks, the population is strongly reduced in winter, in particular when conditions are extremely severe, like in the above-mentioned year in this study. In contrast, Culicidae

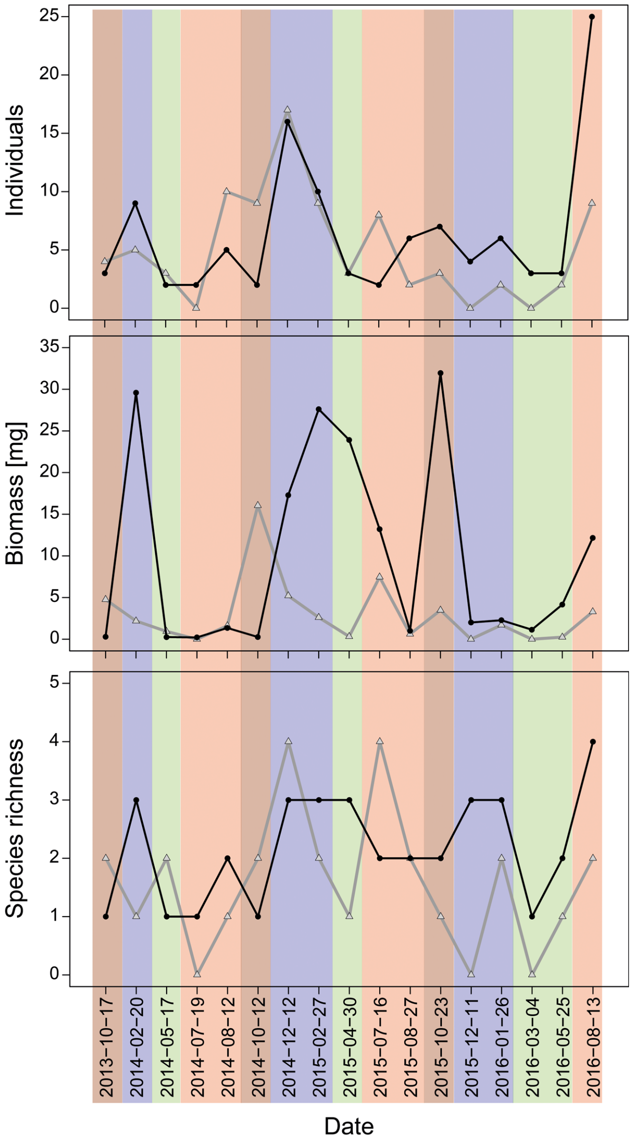

Fig. 5. The numbers, biomass and species richness of the insects recorded in the samples collected from the tree holes at 10.2 m (black symbol and line) and 20.2 m (grey symbol and line) above the ground on each date sampled between 2013 and 2016. Colours indicate the season (brown: autumn, blue: winter, green: spring, red: summer).

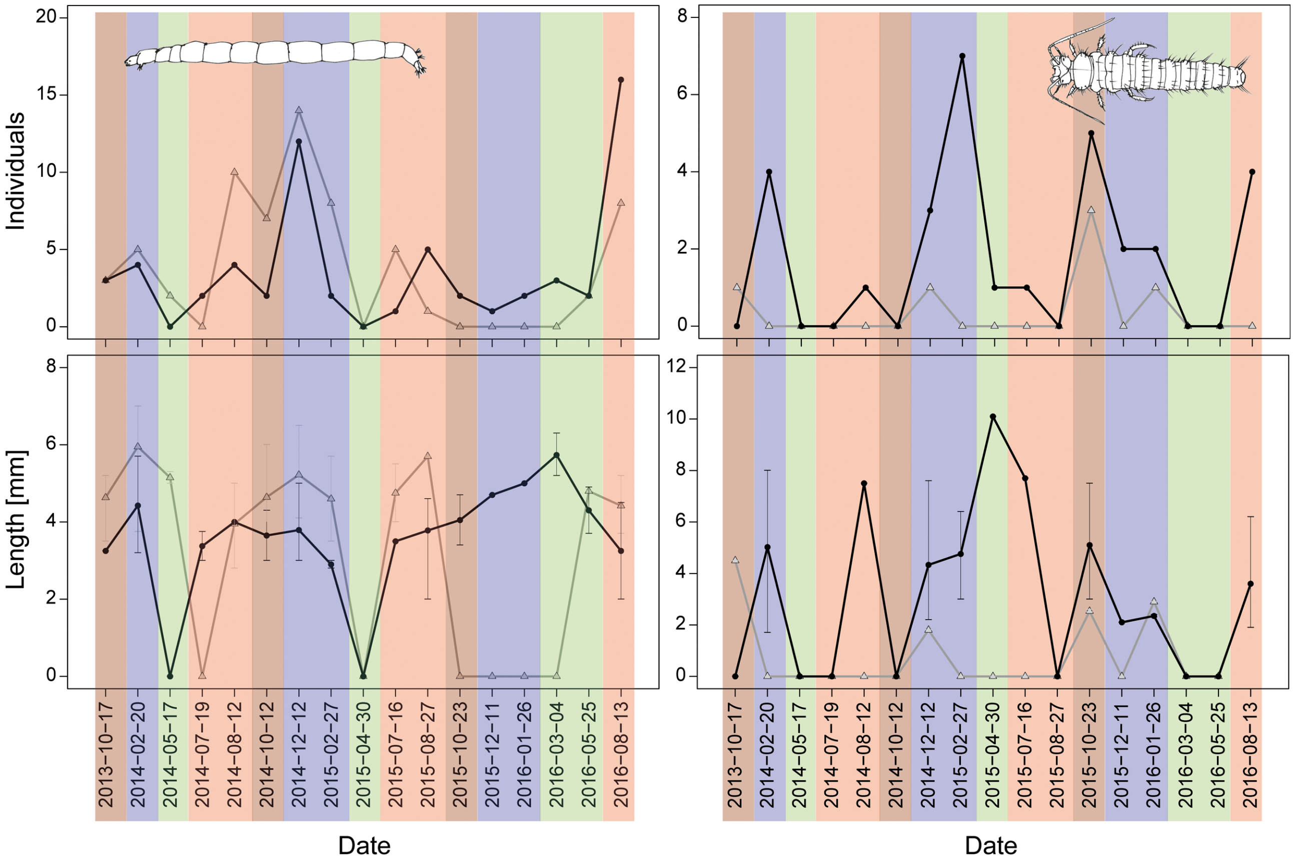

Fig. 6. Total number of individuals and average body length (mean, Min–Max) of *Metriocnemus cavicola* (Diptera: Chironomidae; left) and *Prionocyphon serricornis* (Coleoptera: Scirtidae; right) in the 15 ml samples of water collected from the tree holes at 10.2 m (black symbol and line) and 20.2 m (grey symbol and line) above the ground on each date sampled between 2013 and 2016. Colours indicate the season (brown: autumn, blue: winter, green: spring, red: summer).

have very low frost resistance and overwinter in either the egg or adult stage. Thus, species-specific traits influence their phenology.

Interestingly, the communities in the upper tree hole lagged behind those in the lower tree hole in the present study. This might indicate that the insects first colonize those microhabitats that are less likely to dry out. In contrast, species that are less drought sensitive, such as *Dashylea* sp., *M. cavicola* and *M. florea* (Srivastava, 2005; Dominiak, 2012), might be avoiding competition by colonizing tree holes in the upper canopy.

Seasonality of the most abundant species

M. cavicola and *P. serricornis* were by far the most abundant species in the present study. Both species were also among the most abundant species in previous studies of communities in water-filled tree holes in Central European forests (Kitching, 1971; Schmidl et al., 2008; Gossner et al., 2016). In the present study, both species showed a clear periodicity that was stronger in the upper canopy, most likely due to the more frequent drying out of the upper tree hole. For both species, Kitching (1971, 1972a, 2000) also found a clear seasonal pattern during a one-year study in Great Britain. In agreement with the studies of Kitching, *M. cavicola* occurred year round in the present study, and the simultaneous occurrence of different larval stages indicates multiple colonization events. However, the high numbers in summer reported by Kitching (2000) were not recorded in the present study. Depending on the year, abundances and average body sizes peaked in summer or winter, indicating a non-seasonal periodicity. Interestingly, *Dashylea* sp., which occupy an ecological niche similar to that of *M. cavicola*, peaked in abundance at times when the abundance of *M. cavicola* was low. Moreover, species that are less drought sensitive occurred mainly in the upper tree hole and might thereby avoid competition. These findings might indicate co-evolved temporal competitive exclusion, as proposed by Kitching (1969, 2000) and Barrera (1996). More comprehensive studies on the phenology of these species, including exclusion experiments, are necessary to clarify whether competitive exclusion is a driver of tree hole community dynamics.

Abundance and body size of *P. serricornis* (drought sensitive) in the upper hole lagged behind the values recorded for this species in the lower hole. For *M. cavicola* (drought resistant), this pattern was only observed for abundance, while body size in the lower hole lagged behind that in the upper hole. This result probably indicates that both species colonize holes in the lower canopy first, most likely a consequence of their searching for the most suitable habitats. The probability of finding suitable microhabitats decreases with height in trees because of decreasing availability of their microhabitat. In the study of Gossner et al. (2016), one to three times more tree holes occurred in the canopy (> 2 m height) than near the ground, but the average height was 4 m in both of the regions studied. However, *M. cavicola* seems to mainly colonize tree holes high in the canopy (greater overall abundance was recorded in the upper tree hole) where they develop faster, most likely due to the higher water temperatures in these more exposed holes. Therefore, the body size of this species in the lower tree hole lagged behind that recorded in upper tree hole.

CONCLUSIONS

This three year study on the phenology of insect larvae in water-filled tree holes revealed some interesting patterns that should followed up in the future by studies at larger spatial scales, including experimental work. Based on the results presented and those of previous studies, the following hypotheses are proposed and should be tested in the future: (1) the periodicity of insect communities in water-filled tree holes in Central European forests is independent of season and driven by irregular drying out of tree holes; (2) within canopies, species richness in water-filled tree holes decreases with height above ground due to the harsher conditions prevailing in the upper canopy; (3) differences in microclimate result in different temporal dynamics in the lower and upper canopy; and (4) competitive exclusion is an important structuring mechanism of the communities of insect larvae inhabiting water-filled tree holes.

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Supplement S1: Details of the stand and tree studied.
Supplement S2: Periodicity in the numbers of rare species.
Supplement S3: Details of time series analyses.
Supplement S4: Data on the species of insects collected.
Supplement S5: Raw species data.