Supplementary material to:

GOSSNER M.M. 2018: A three year study of the phenology of insect larvae (Coleoptera, Diptera) in water-filled tree holes in the canopy of a beech tree. — *Eur. J. Entomol.* **115**: 524–534.

Content:

Supplement S1: Details of stand and studied tree studied

Figure S1-1: Photographs of the beech tree sampled in a mixed broad-leaf forest in southern Bavaria, Germany. A–C: Tree studied in autumn (12.10.2014), winter with snow (13.02.2015) and winter without snow (12.12.2014). D: View of the surrounding forest canopy from the top of the tree (23.10.2015). E: The high-resolution Thermochron® iButton® DS1921Z-F5 in a water-proof protective capsule DS9107 used to measure temperature in water-filled tree holes. F: The lower (10.2 m height) and G: The upper (20.2 m) tree hole on 04.03.2016. H, I: The tree was climbed once every two months using the single-rope climbing technique between October 2013 and August 2016 to take water samples and extract temperature data from the logger (20.04.2014). J: View from the canopy in the southern part of the forest where oak trees in the surrounding area were harvested in winter 2014/2015 (27.02.2015). K: View from the ground of the canopy of the tree studied (23.10.2015). Photograph credits: H, I Jana Petermann, others Martin M. Gossner.

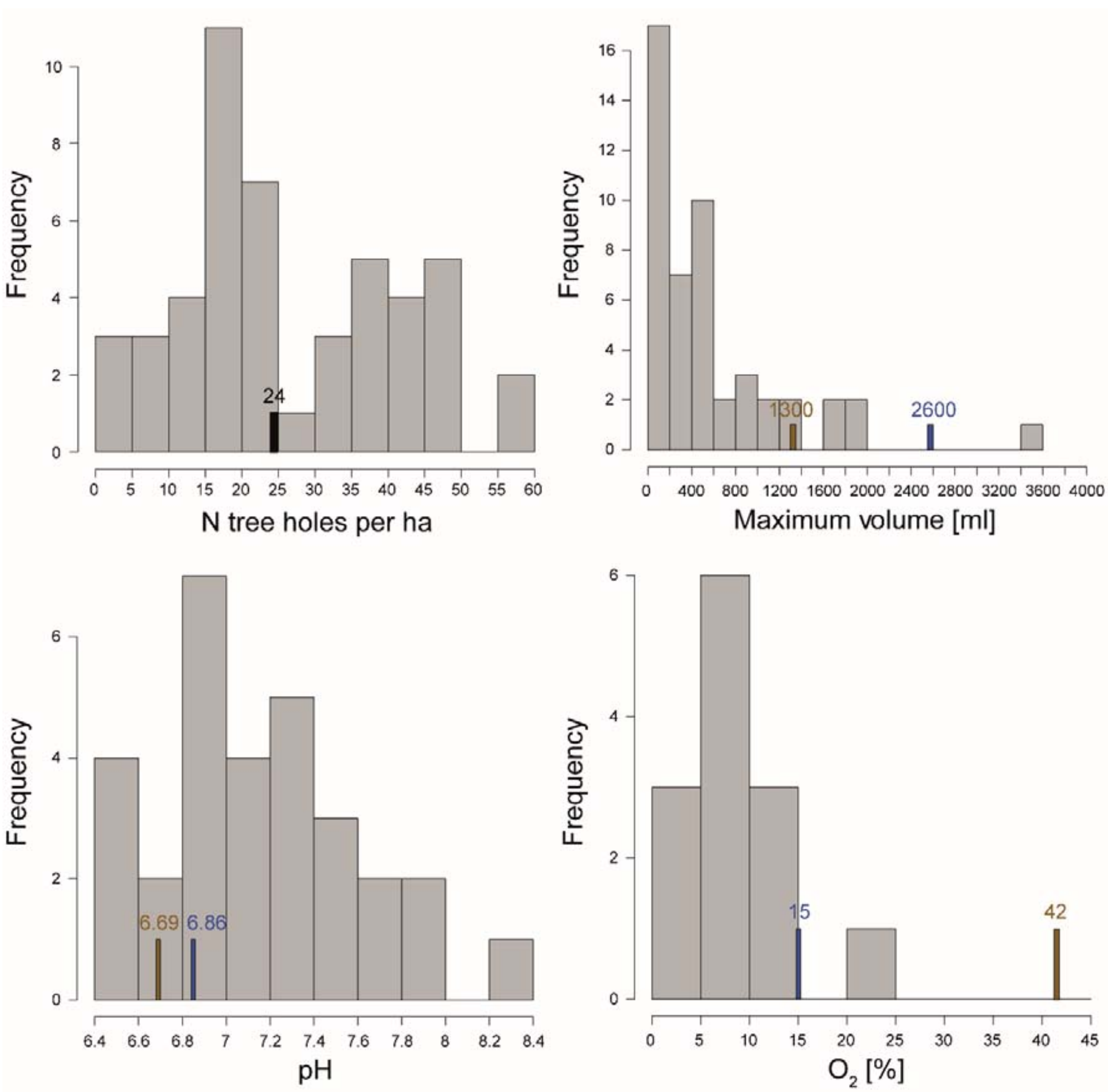

Figure S1-2: Histograms showing the frequencies of the number of tree holes per hectare (top left), maximum volume of tree holes (top right), pH (bottom left), and oxygen saturation (bottom right) of natural tree holes in the canopy of mature beech trees recorded by Gossner *et al.* (2016) (Total: Schwäbische Alb 26, Hainich-Dün 22). The coloured bars and numbers show values recorded in the present study (yellow: lower tree hole 10 m above the ground, blue: upper tree hole 20 m above the ground).

Reference

Gossner M.M., Lade P., Rohland A., Sichardt N., Kahl T., Bauhus J., Weisser W.W. & Petermann J.S. 2016: Effects of management on aquatic tree-hole communities in temperate forests are mediated by detritus amount and water chemistry. — *J. Anim. Ecol*. **85**: 213–226.

Supplement S2: Periodicity in the numbers of the rare species

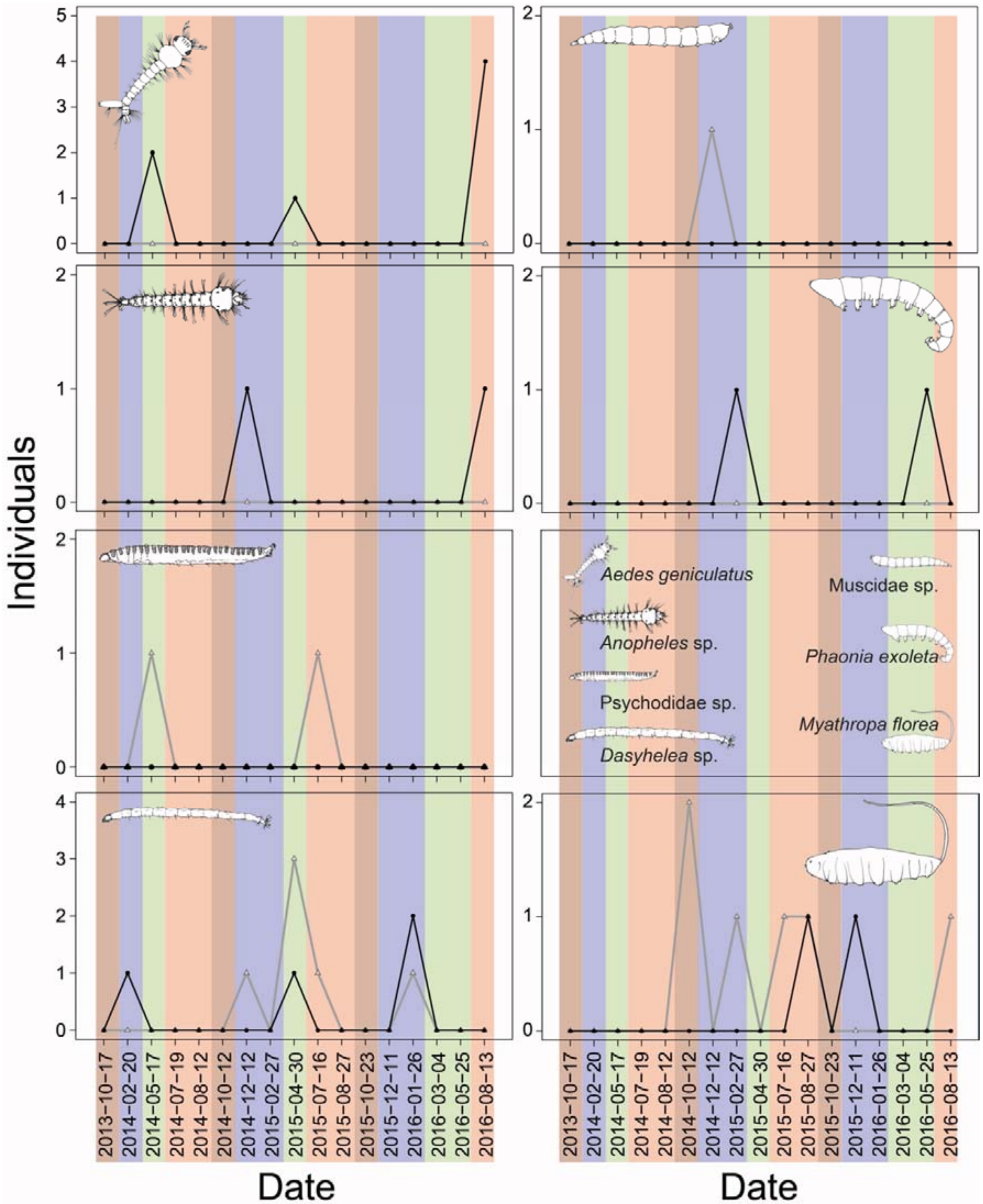

Figure S2-1: Number of individuals of *Aedes geniculatus*, *Anopheles* sp. (both Diptera: Culicidae; left), Psychodidae (Diptera), *Dasyhlea* sp. (Diptera: Ceratopogonidae), *Muscidae* sp., *Phaonia exoleta* (both Diptera: Muscidae) and *Myathropa florea* (Diptera: Syrphidae) in 15 ml samples of the water from the tree holes at 10.2 m (black symbol and line) and 20.2 m (grey symbol and line) above the ground collected on each date sampled between 2013 and 2016. Colours indicate the season (brown: autumn, blue: winter, green: spring, red: summer).

Supplement S3: Details of time series analyses

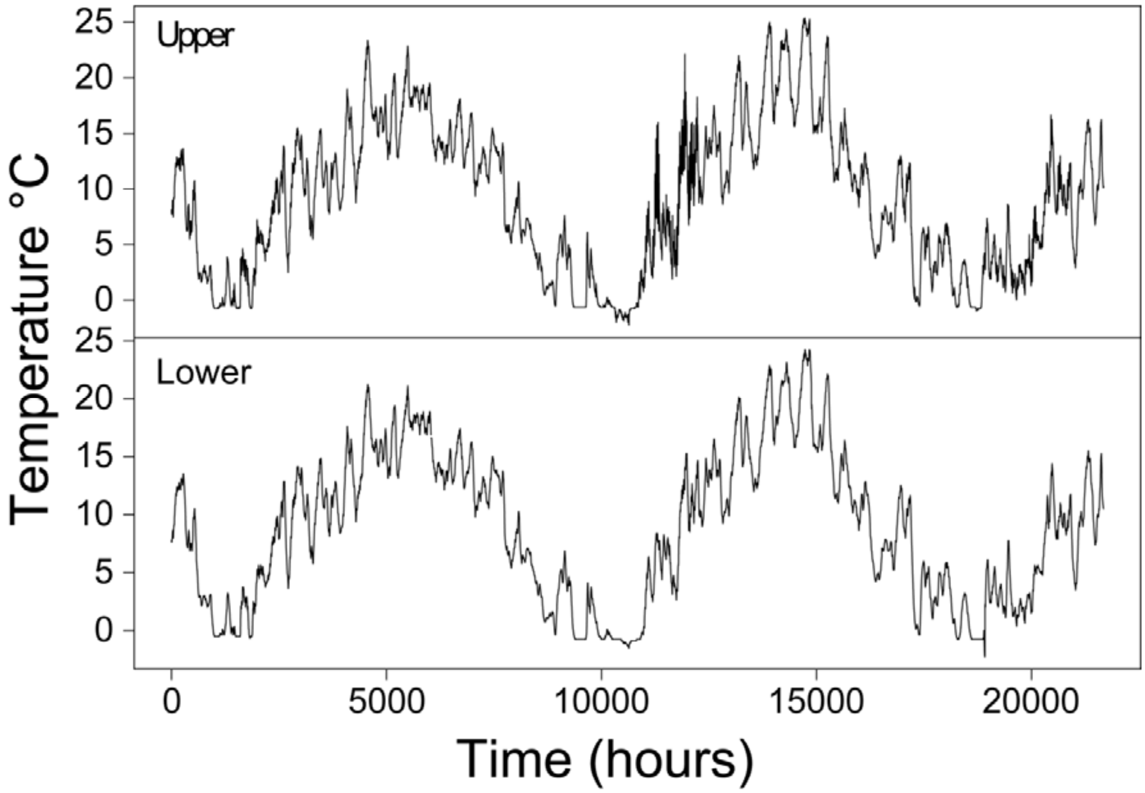

Figure S3-1: Water temperature recorded in the upper and lower tree holes during the period from October 2013 to May 2016.

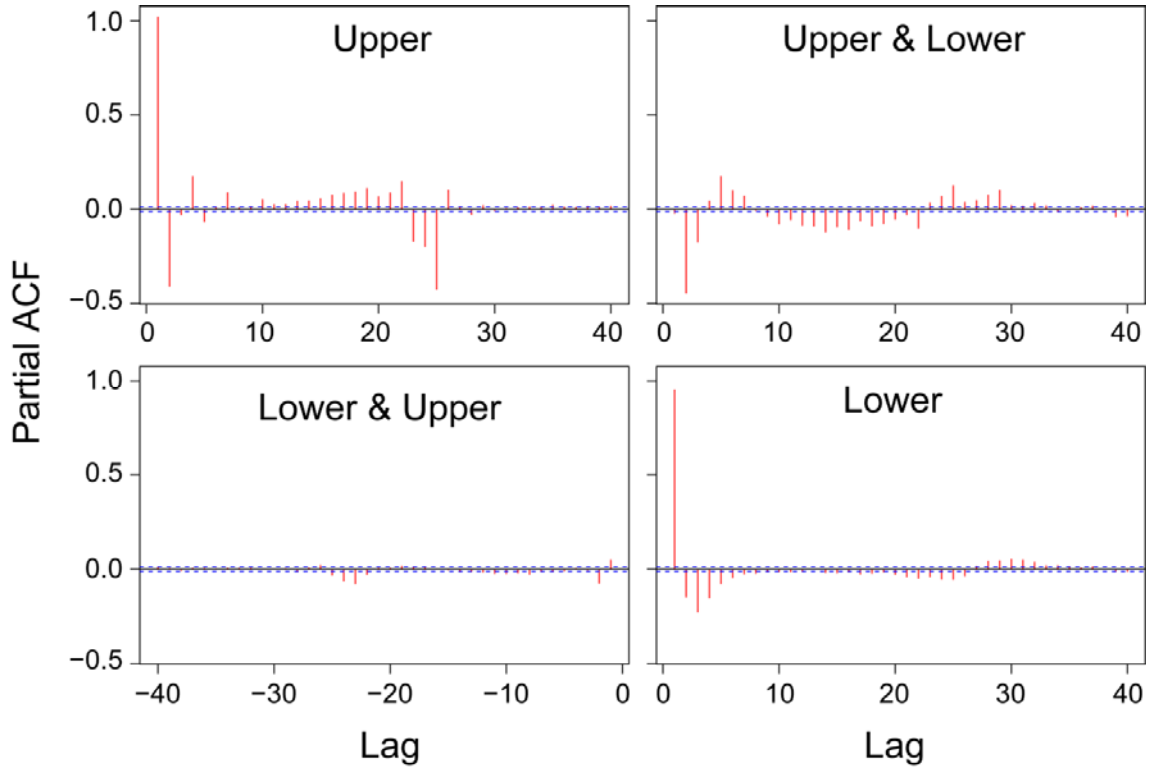

Figure S3-2: Partial autocorrelation (ACF=Autocorrelation function) between the mean hourly water temperature in the upper (top-left panel) and lower (bottom-right) tree holes and cross-correlation between the upper and lower tree holes (top-right, bottom-left) throughout the period from October 2013 to May 2016. The lag is plotted in units of time and not number of observations. When the vertical red bars cross the horizontal blue dashed lines, the partial autocorrelation at this lag is significant. The top-left and bottom-right panels indicate significant periodicity in the temperature of the water in the upper and lower holes (day vs. night as well as seasonal peaks, see Fig. S1-1), with larger peaks in the upper hole. This suggests that the upper hole is less buffered against changes in air temperature than the lower hole. Significant cross-correlation between the upper and lower holes (top-right panel) indicate that positive changes in the upper hole are associated with negative changes in the lower hole and vice versa. This indicates that temperature of the water in the lower hole lags behind that in the upper hole, which is another indication of a greater buffering against air temperature in the lower hole.

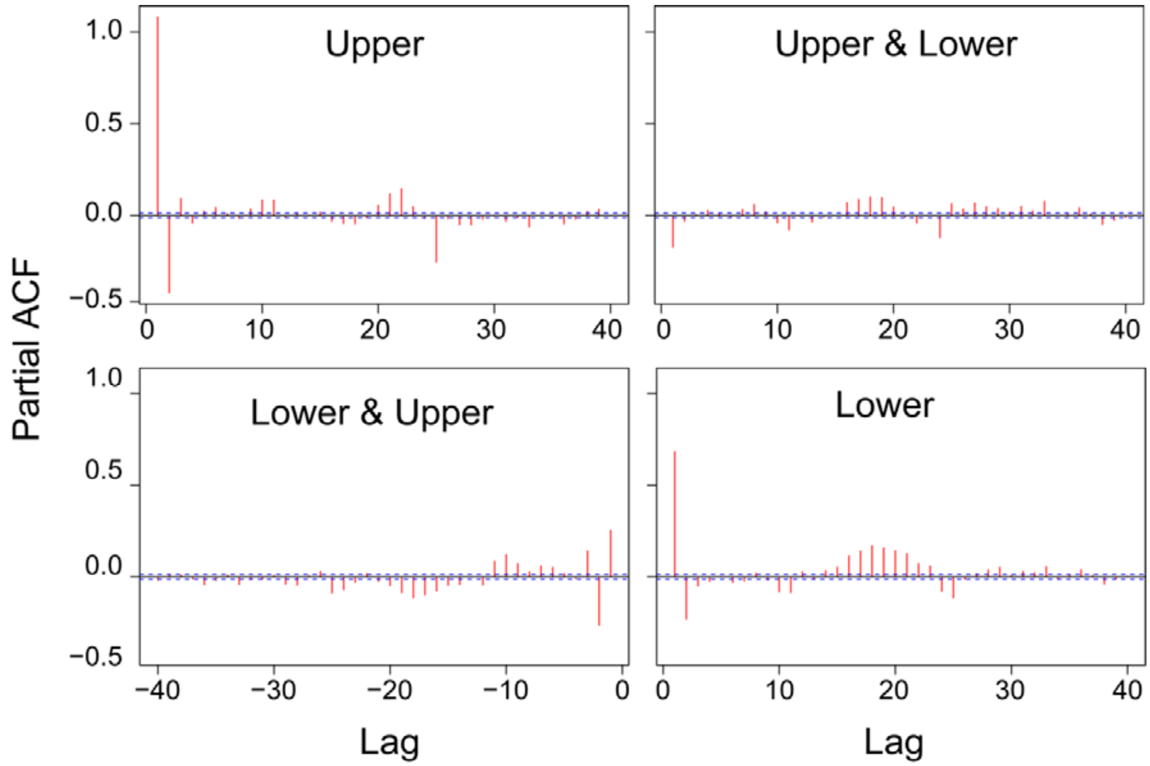

Figure S3-3: Partial autocorrelation (ACF=autocorrelation function) between the differences in the mean hourly temperature of the water and air temperature in the upper (top-left panel) and lower (bottom-right) tree hole and cross-correlation between the upper and lower tree holes (top-right, bottom-left) throughout the period from October 2013 to May 2016. The lag is plotted in units of time and not number of observations. When the vertical red bars cross the horizontal blue dashed lines, the partial autocorrelation at this lag is significant. The larger peaks at lag 1 and 2 for the upper hole compared with the lower hole indicate a greater buffering against changes in air temperature in the lower hole.

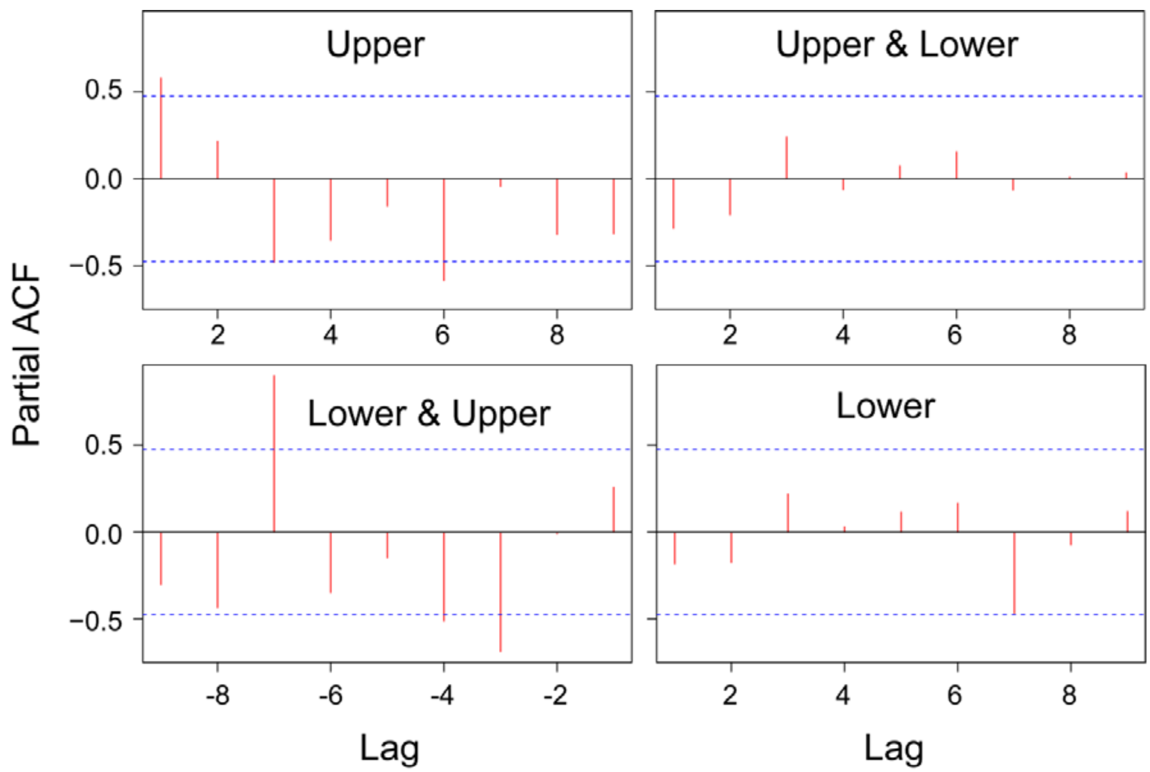

Figure S3-4: Partial autocorrelation (ACF=autocorrelation function) between the number of insect larvae per 15 ml water in the upper (top-left panel) and lower (bottom-right) tree holes and cross-correlation between the upper and lower tree hole (top-right, bottom-left) based on 17 samples collected between October 2013 and August 2016. The lag is plotted in units of time and not number of observations. When the vertical red bars cross the horizontal blue dashed lines, the partial autocorrelation at this lag is significant. The evidence for periodicity is stronger for the upper than the lower hole: the partial autocorrelation is significant at lag 1 (positive) and at lags 3 and 6 (negative) for the upper hole (top-left) but only significant and negative at lag 7 for the lower hole (bottom-right). The significant cross-correlation between the lower and upper hole (bottom-left) indicates that positive changes in the lower hole are associated with positive changes in the upper hole at lag 7. In contrast, positive changes in the lower hole at lags 3 and 4 are associated with negative changes in the upper hole and vice versa. The communities in the upper hole seem to lag behind those in the lower hole, as indicated by a significant cross-correlation between Lower & Upper, but not between Upper & Lower.

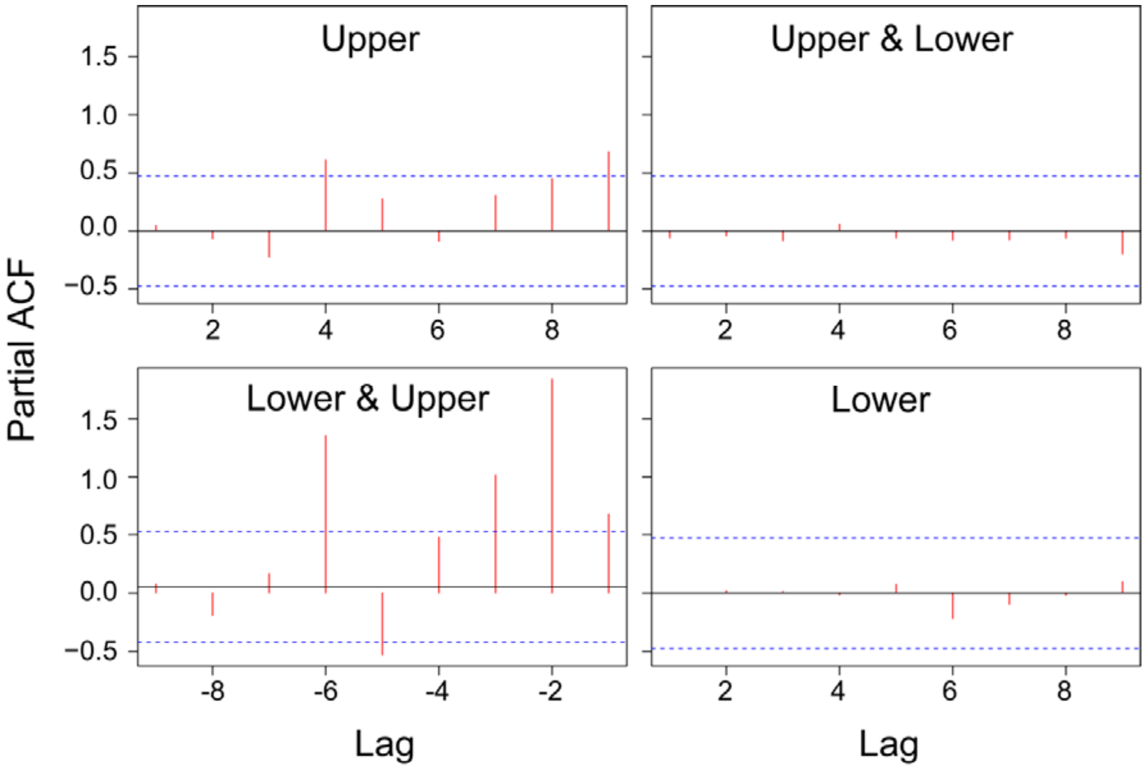

Figure S3-5: Partial autocorrelation (ACF=autocorrelation function) between the biomass of insect larvae per 15 ml of water in the upper (top-left panel) and lower (bottom-right) tree holes and cross-correlation between the upper and lower tree holes (top-right, bottom-left) based on 17 samples collected between October 2013 and August 2016. The lag is plotted in units of time and not number of observations. When the vertical red bars cross the horizontal blue dashed lines, the partial autocorrelation at this lag is significant. Similar to the number of insect larvae (Fig. S1-4), the evidence for periodicity in the biomass of insects is stronger for the upper than the lower hole: the partial autocorrelation is significant and positive at lag 4 and lag 9 for the upper hole (top-left) but not for the lower hole (bottom-right). The significant cross-correlation between Lower & Upper (bottom left) indicates that positive changes in the lower hole are associated with positive changes in the upper hole at lags 1, 2, 3 and 6. In contrast, positive changes in the lower hole at lag 5 are associated with negative changes in the upper hole and vice versa. The community in the upper hole seem to lag behind that in the lower hole, as indicated by a significant cross-correlation between Lower & Upper, but not between Upper & Lower.

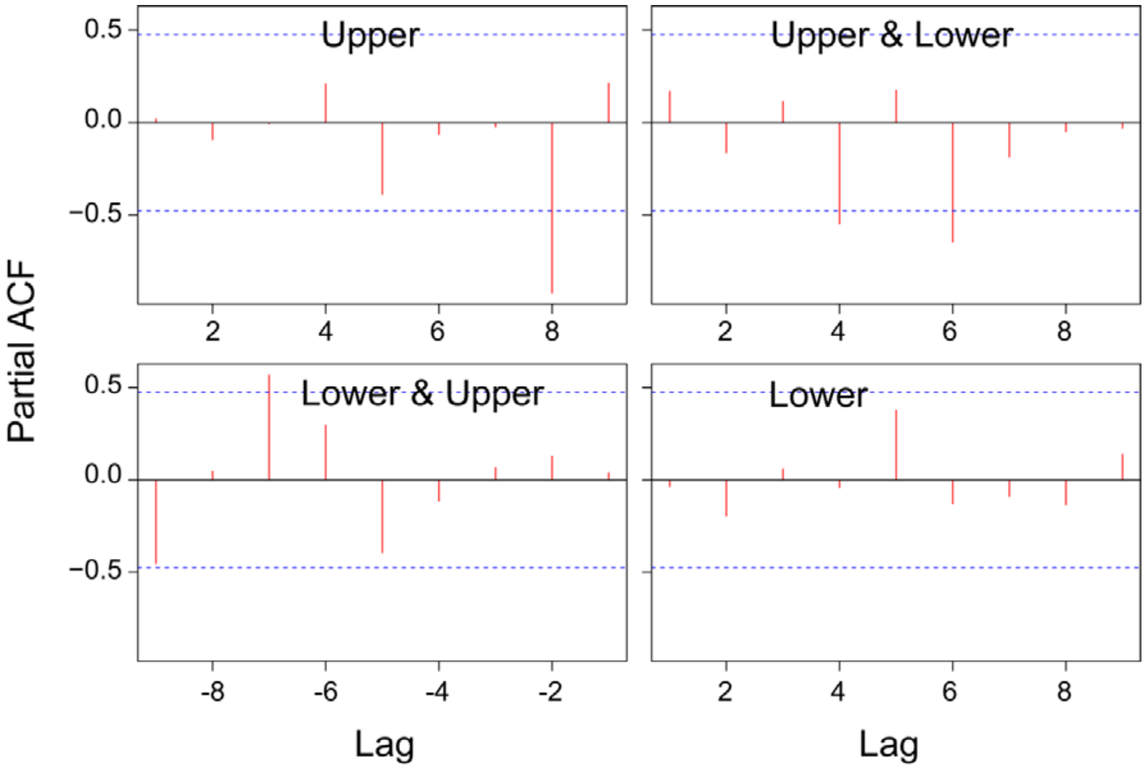

Figure S3-6: Partial autocorrelation (ACF=autocorrelation function) between insect larvae species richness per 15 ml of water in the upper (top-left panel) and lower (bottom-right) tree holes and cross-correlation between the upper and the lower tree holes (top-right, bottom-left) based on 17 samples taken between October 2013 and August 2016. The lag is plotted in units of time and not number of observations. When the vertical red bars cross the horizontal blue dashed lines, the partial autocorrelation at this lag is significant. The evidence for periodicity is stronger for the upper than the lower hole: the partial autocorrelation is significant and negative at lag 8 for the upper hole (top-left) but not for the lower hole (bottom-right). The significant cross-correlation between Upper & Lower (top-right) and Lower & Upper (bottom-left) indicate that positive changes in the upper hole are associated with negative changes in the lower hole and vice versa at lags 4 and 6, and that positive changes in the lower hole are associated with positive changes in the upper hole at lag 7. The community in the upper hole seem to lag behind that in the lower hole at some point (significant cross-correlation for Lower & Upper) but differ at other points (significant cross-correlation for Upper & Lower).

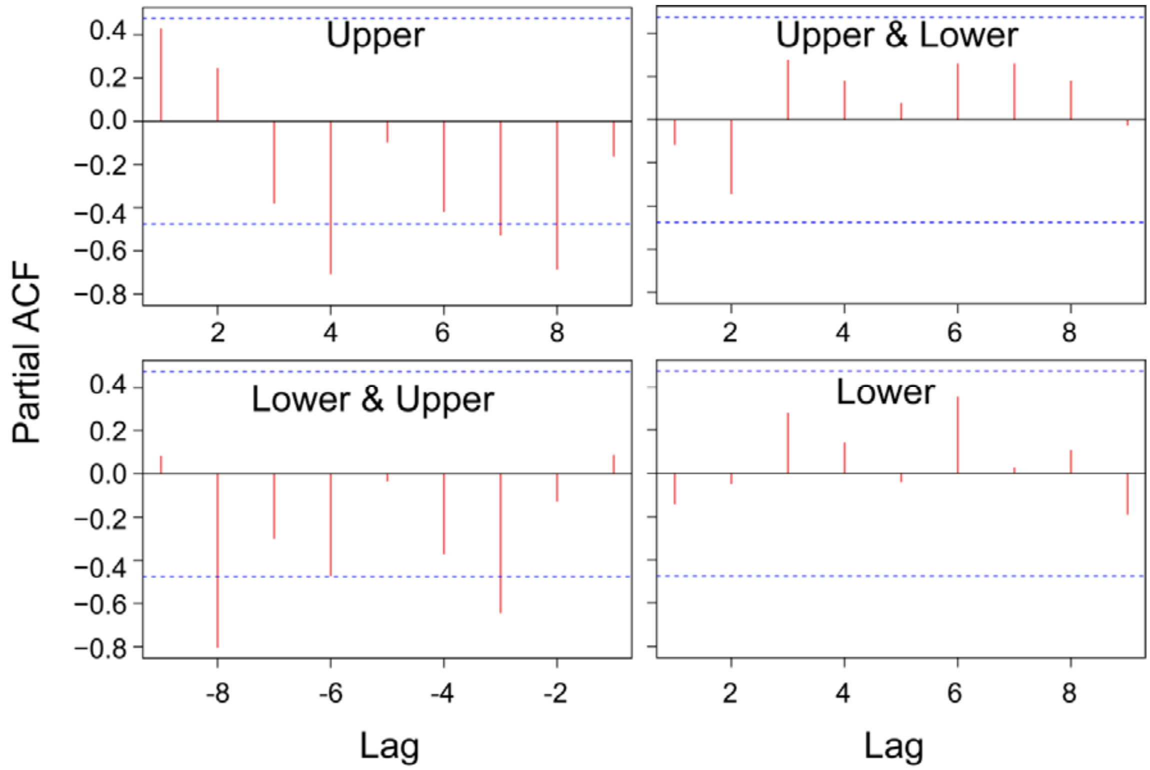

Figure S3-7: Partial autocorrelation (ACF=autocorrelation function) between number of individuals of *Metriocnemus cavicola* per 15 ml of water in the upper (top-left panel) and lower (bottom-right) tree holes and cross-correlation between the upper and the lower tree holes (top-right, bottom-left) based on 17 samples collected between October 2013 and August 2016. The lag is plotted in units of time and not number of observations. When the vertical red bars cross the horizontal blue dashed lines, the partial autocorrelation at this lag is significant. The evidence for periodicity is stronger for the upper than in the lower hole: the partial autocorrelation is significant and negative at lags 4, 7 and 8 for the upper hole (top-left) but not for the lower hole (bottom-right). The significant cross-correlation between Lower & Upper (bottom-left) indicates that positive changes in the lower hole are associated with negative changes in the upper hole and vice versa at lags 3, 6 and 8. The abundances in the upper hole seem to lag behind those in the lower hole, as indicated by the significant cross-correlation between Lower & Upper, but not between Upper & Lower.

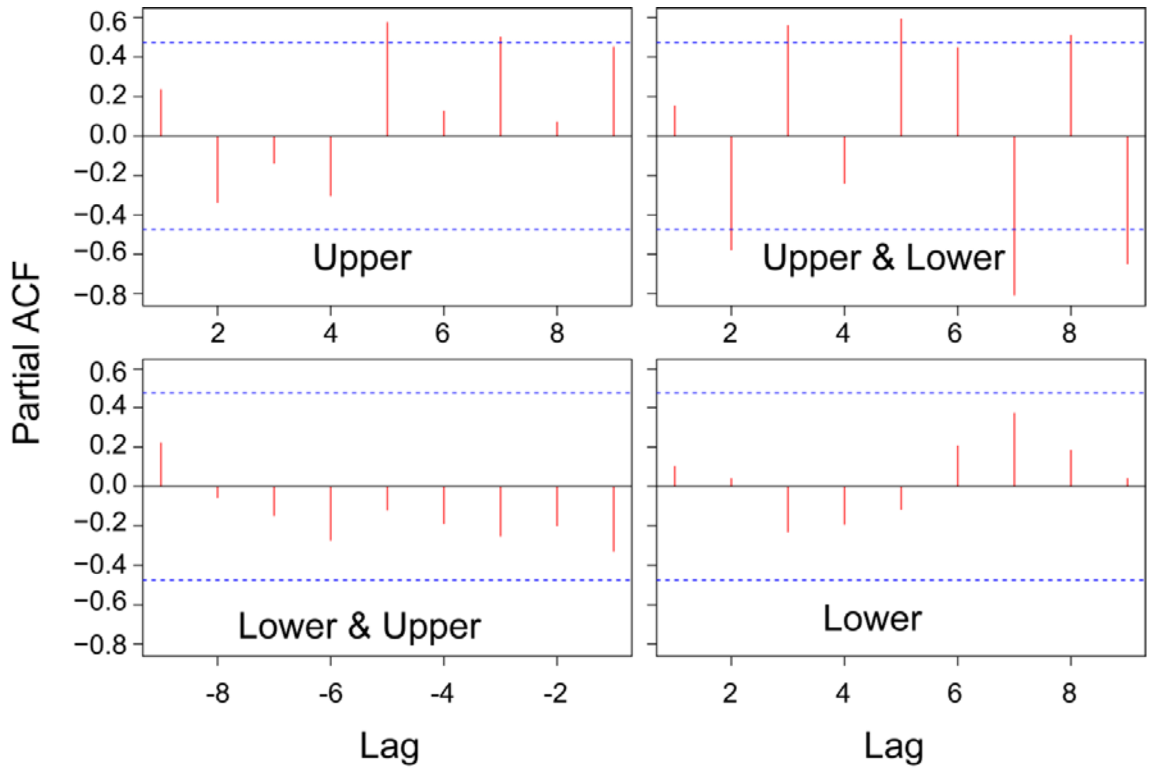

Figure S3-8: Partial autocorrelation (ACF=autocorrelation function) between mean body length of *Metriocnemus cavicola* in the upper (top-left panel) and the lower (bottom-right) tree holes and cross-correlation between the upper and the lower tree holes (top-right, bottom-left) based on 17 samples collected between October 2013 and August 2016. The lag is plotted in units of time and not number of observations. When the vertical red bars cross the horizontal blue dashed lines, the partial autocorrelation at this lag is significant. The evidence for periodicity is stronger for the upper than in the lower hole: the partial autocorrelation is significant and positive at lags 5 and 7 for the upper hole (top-left) but not for the lower hole (bottom-right). The significant cross-correlation between Upper & Lower (top-right) indicates that positive changes in the upper hole are associated with positive changes in the lower hole at lags 3, 5 and 8 but with negative changes in the lower hole at lags 2, 7 and 9 and vice versa. The mean body sizes in the lower hole seem to lag behind those in the upper hole, as indicated by the significant cross-correlation between Upper & Lower, but not between Lower & Upper.

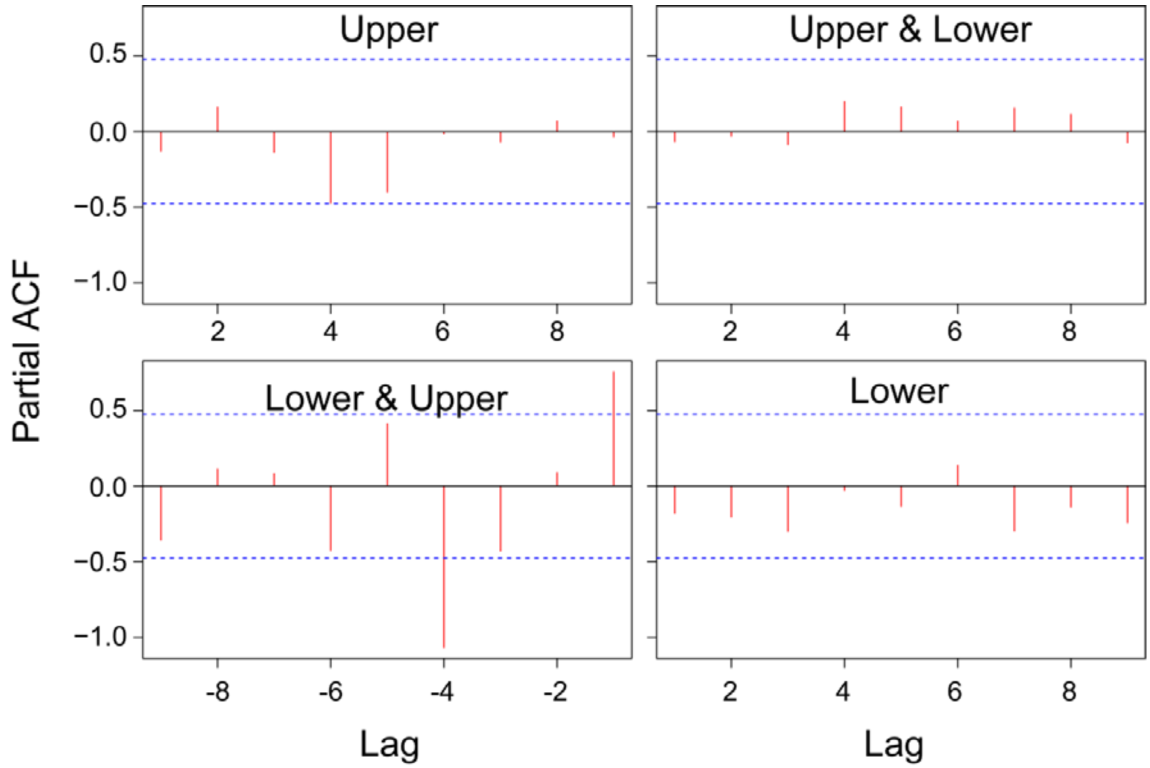

Figure S3-9: Partial autocorrelation (ACF=autocorrelation function) between number of individuals of *Prionocyphon serricornis* per 15 ml of water in the upper (top-left panel) and lower (bottom-right) tree holes and cross-correlation between the upper and the lower tree holes (top-right, bottom-left) based on 17 samples collected between October 2013 and August 2016. The lag is plotted in units of time and not number of observations. When the vertical red bars cross the horizontal blue dashed lines, the partial autocorrelation at this lag is significant. The evidence for periodicity is stronger for the upper than in the lower hole: the partial autocorrelation is significant and negative at lag 4 for the upper hole (top-left) but not for the lower hole (bottom-right). The significant cross-correlation between Lower & Upper (bottom-left) indicates that positive changes in the lower hole are associated with positive changes in the upper hole at lag 1 but with negative changes in the upper hole at lag 4 and vice versa. The abundances in the upper hole seem to lag behind those recorded in the lower hole, as indicated by the significant cross-correlation between Lower & Upper, but not between Upper & Lower.

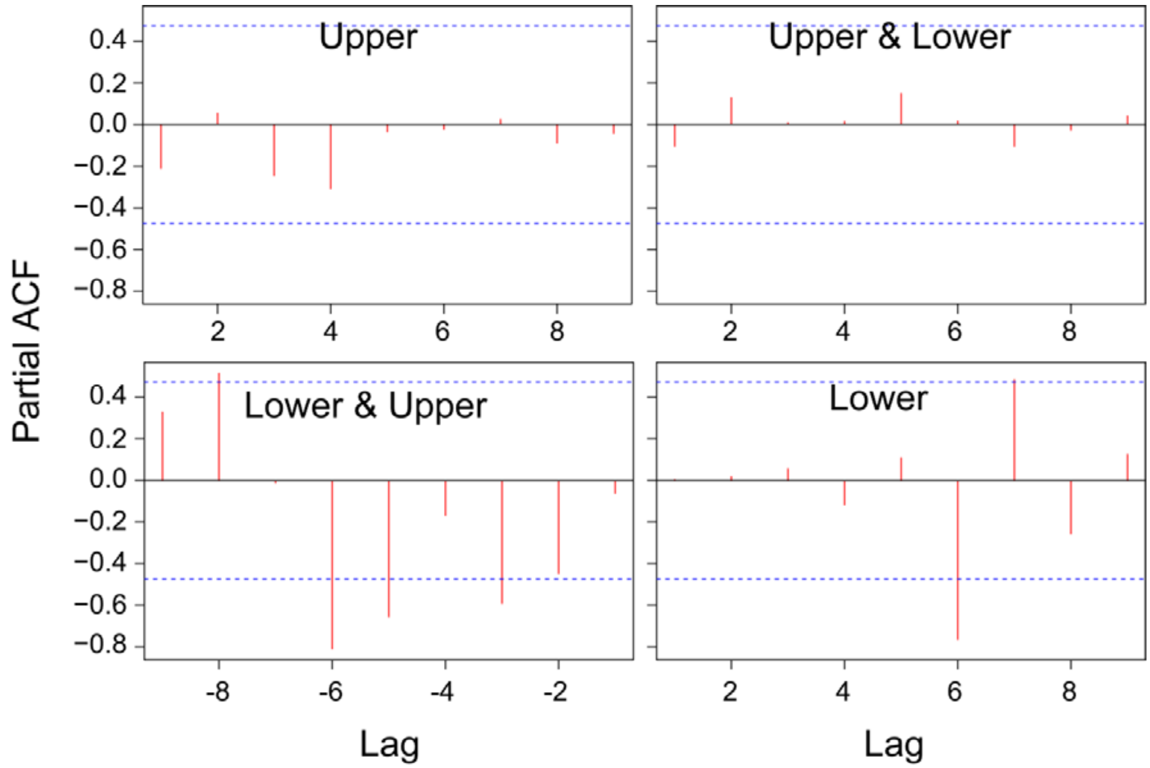

Figure S3-10: Partial autocorrelation (ACF=autocorrelation function) between mean body length of *Prionocyphon serricornis* in the upper (top-left panel) and lower (bottom-right) tree holes and cross-correlation between the upper and the lower tree holes (top-right, bottom-left) based on 17 samples collected between October 2013 and August 2016. The lag is plotted in units of time and not number of observations. When the vertical red bars cross the horizontal blue dashed lines, the partial autocorrelation at this lag is significant. The evidence for periodicity is stronger for the lower than in the upper hole: the partial autocorrelation is significant and negative at lag 6 and positive at lag 7 for the lower hole (bottom-right) but not for the upper hole (top-left). The significant cross-correlation between Lower & Upper (bottom-left) indicates that negative changes in the lower hole are associated with negative changes in the upper hole at lags 3, 5 and 6 but with positive changes in the upper hole at lag 8 and vice versa. The mean body sizes in the upper hole seem to lag behind those recorded in the lower hole, as indicated by the significant cross-correlation between Lower & Upper, but not between Upper & Lower.

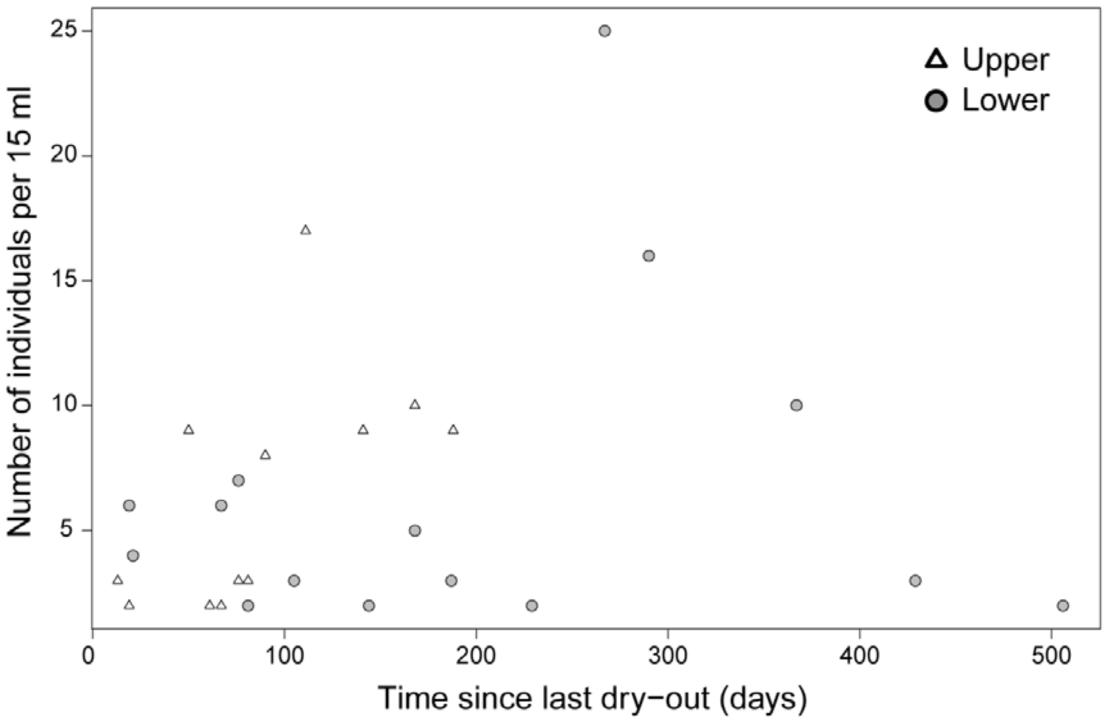

Figure S3-11: Number of individuals in a 15 ml sample of water from the upper (top) and lower (bottom) tree holes as a function of time since they last dried out. There is a weak indication of an increase in insect larval abundance with increase in time since a hole last dried out. Please note that different data points come from the same two tree holes at different times, seasons and years.

Supplement S4: Data on the species of insects sampled

Table S4-1: Details of the communities (Ind= number of Individuals, SR=Species Richness, Biomass) and size (Min, Mean, Max body length) of the two most abundant species recorded in the two tree holes (L=lower, U=Upper) in a beech tree in Southern Bavaria, Germany from 2013 to 2016. On each date, a 15 ml water sample was taken from each tree hole. Additionally, the season (A=Autumn, Sp=Spring, Su=Summer, W=Winter) and water volume in the tree holes at the time of sampling and the time in days since they last dried out are given. AedeGeni=*Aedes geniculatus*, Anop=*Anopheles* sp., Dash=*Dasyhelea* sp., MetrCavi=*Metriocnemus cavicola*, Musc=*Muscidae* sp., MyatFlor=*Myathropa florea*, PhaoExco=*Phaonia excoleta*, PrioSerr=*Prionocyphon serricornis*.

Date	Season	Position	Aede Geni	Anop	Dash	Metr Cavi	Musc	Myat Flor	Phao Exco	Prio Serr	Psychodidae	Ind	SR	Biomass	Water volume	Metr Mean	Metr Max	Metr Min	Prio Mean	Prio Max	Pri Min	Time Since Last Dried Out
17.10.2013	A	L	0	0	0	3	0	0	0	0	0	3	1	0.28	3675	3.25	3.25	3.25	0.00	0.00	0.00	NA
17.10.2013	A	U	0	0	0	3	0	0	0	1	0	4	2	4.77	2925	4.63	5.20	3.50	4.50	4.50	4.50	NA
20.02.2014	W	L	0	0	1	4	0	0	0	4	0	9	3	29.58	1859	4.43	5.70	3.20	5.03	8.00	1.70	NA
20.02.2014	W	U	0	0	0	5	0	0	0	0	0	5	1	2.19	50	5.95	7.00	3.75	0.00	0.00	0.00	NA
17.05.2014	Sp	L	2	0	0	0	0	0	0	0	0	2	1	0.25	3675	0.00	0.00	0.00	0.00	0.00	0.00	81
17.05.2014	Sp	U	0	0	0	2	0	0	0	0	1	3	2	0.94	2925	5.15	5.30	5.00	0.00	0.00	0.00	81
19.07.2014	Su	L	0	0	0	2	0	0	0	0	0	2	1	0.21	2808	3.38	3.75	3.00	0.00	0.00	0.00	144
19.07.2014	Su	U	0	0	0	0	0	0	0	0	0	0	0	0.00	665	0.00	0.00	0.00	0.00	0.00	0.00	144
12.08.2014	Su	L	0	0	0	4	0	0	0	1	0	5	2	1.35	3675	4.00	4.00	4.00	7.50	7.50	7.50	168
12.08.2014	Su	U	0	0	0	10	0	0	0	0	0	10	1	1.61	2925	3.95	5.00	2.80	0.00	0.00	0.00	168
12.10.2014	A	L	0	0	0	2	0	0	0	0	0	2	1	0.26	2652	3.65	4.30	3.00	0.00	0.00	0.00	229
12.10.2014	A	U	0	0	0	7	0	2	0	0	0	9	2	16.05	1380	4.64	6.00	3.00	0.00	0.00	0.00	50
12.12.2014	W	L	0	1	0	12	0	0	0	3	0	16	3	17.29	2208	3.79	5.00	3.00	4.33	7.60	2.20	290
12.12.2014	W	U	0	0	1	14	1	0	0	1	0	17	4	5.21	2925	5.22	6.50	4.10	1.80	1.80	1.80	111
27.02.2015	W	L	0	0	0	2	0	0	1	7	0	10	3	27.61	3500	2.90	3.00	2.80	4.76	6.40	3.00	367
27.02.2015	W	U	0	0	0	8	0	1	0	0	0	9	2	2.61	2925	4.60	5.70	3.50	0.00	0.00	0.00	188

30.04.2015	Sp	L	1	0	1	0	0	0	0	1	0	3	3	23.92	2208	0.00	0.00	0.00	10.10	10.10	10.10	429
30.04.2015	Sp	U	0	0	3	0	0	0	0	0	0	3	1	0.32	2184	0.00	0.00	0.00	0.00	0.00	0.00	13
16.07.2015	Su	L	0	0	0	1	0	0	0	1	0	2	2	13.20	1936	3.50	3.50	3.50	7.70	7.70	7.70	506
16.07.2015	Su	U	0	0	1	5	0	1	0	0	1	8	4	7.46	675	4.75	5.50	4.00	0.00	0.00	0.00	90
27.08.2015	Su	L	0	0	0	5	0	1	0	0	0	6	2	1.01	2964	3.78	4.60	2.00	0.00	0.00	0.00	19
27.08.2015	Su	U	0	0	0	1	0	1	0	0	0	2	2	0.62	1794	5.70	5.70	5.70	0.00	0.00	0.00	19
23.10.2015	A	L	0	0	0	2	0	0	0	5	0	7	2	31.95	2484	4.05	4.70	3.40	5.10	7.50	3.00	76
23.10.2015	A	U	0	0	0	0	0	0	0	3	0	3	1	3.48	1656	0.00	0.00	0.00	2.53	2.60	2.50	76
11.12.2015	W	L	0	0	0	1	0	1	0	2	0	4	3	2.01	3675	4.70	4.70	4.70	2.10	2.10	2.10	21
11.12.2015	W	U	0	0	0	0	0	0	0	0	0	0	0	0.00	2925	0.00	0.00	0.00	0.00	0.00	0.00	21
26.01.2016	W	L	0	0	2	2	0	0	0	2	0	6	3	2.27	3675	5.00	5.00	5.00	2.35	2.40	2.30	67
26.01.2016	W	U	0	0	1	0	0	0	0	1	0	2	2	1.70	2925	0.00	0.00	0.00	2.90	2.90	2.90	67
04.03.2016	Sp	L	0	0	0	3	0	0	0	0	0	3	1	1.14	3675	5.73	6.30	5.20	0.00	0.00	0.00	105
04.03.2016	Sp	U	0	0	0	0	0	0	0	0	0	0	0	0.00	2925	0.00	0.00	0.00	0.00	0.00	0.00	105
25.05.2016	Sp	L	0	0	0	2	0	0	1	0	0	3	2	4.16	3675	4.30	4.90	3.70	0.00	0.00	0.00	187
25.05.2016	Sp	U	0	0	0	2	0	0	0	0	0	2	1	0.24	2925	4.80	4.80	4.80	0.00	0.00	0.00	61
13.08.2016	Su	L	4	1	0	16	0	0	0	4	0	25	4	12.16	3500	3.25	4.50	2.00	3.60	6.20	1.90	267
13.08.2016	Su	U	0	0	0	8	0	1	0	0	0	9	2	3.29	2340	4.42	5.20	3.70	0.00	0.00	0.00	141

Figure S4-1: Water-filled tree holes at 10.2 m (A) and 20.2 m (B) above the ground frozen completely in winter (observed on 13.02.2015). Two weeks later (27.02.2015), after being completely frozen for four weeks, the upper 1–3 cm thawed (C–E). While in the upper hole larvae of *Myathropa florea* became frozen in the ice and died (arrows in D, F), *Prionocyphon serricornis* larvae (see arrow in E) survived in the lower hole and were abundant in the 1 cm depth of water. Photograph credits: Martin M. Gossner.

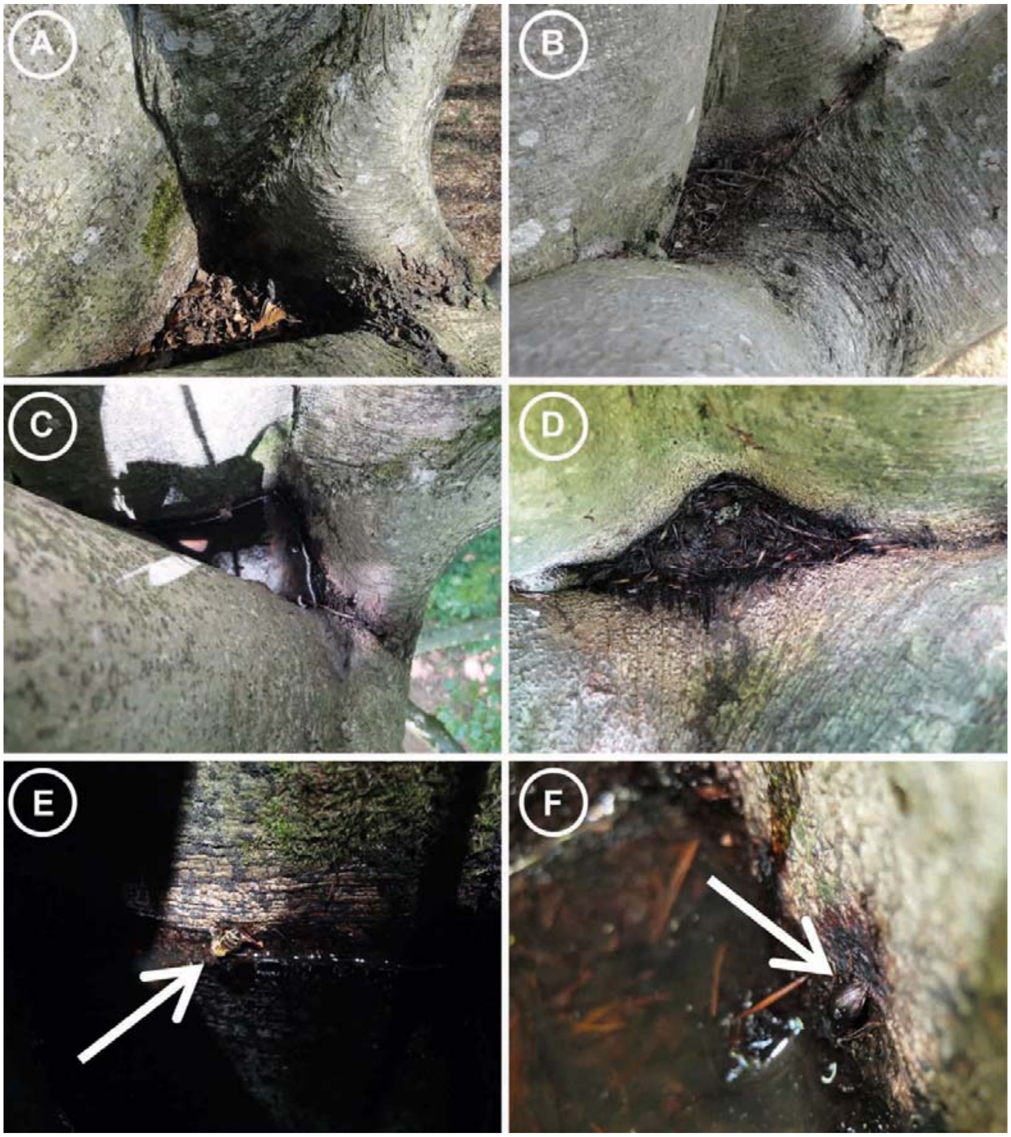

Figure S4-2: Water-filled tree holes at 10.2 m (A) and 20.2 m (B) above the ground that dried out completely on 21 March 2014, before bud burst. On 19 July 2014, the lower tree hole contained almost the maximum volume of water (C), while only moist mud remained in the upper tree hole (D). During this dry period, the lower tree hole was one of the few remaining sources of water for insects, such as honey bees (*Apis mellifera* Linnaeus, 1758) (E) and beetles, e.g., Tenebrionidae *Stenomax aeneus* (Scopoli, 1763) (F) (white arrows). Photograph credits: Martin M. Gossner.

Figure S4-3: The terrestrial pulmonate gastropod mollusc *Lehmannia marginata* (O. F. Müller, 1774) occurs mainly on the trunks of beech trees where it feeds on fungal fruiting bodies and mycelia, and rasps algae and lichens from the bark. It was also frequently observed in the tree holes studied at 10.2 m (A) and 20.2 m (B) above the ground, here on 12 October 2014. Photograph credits: Martin M. Gossner.

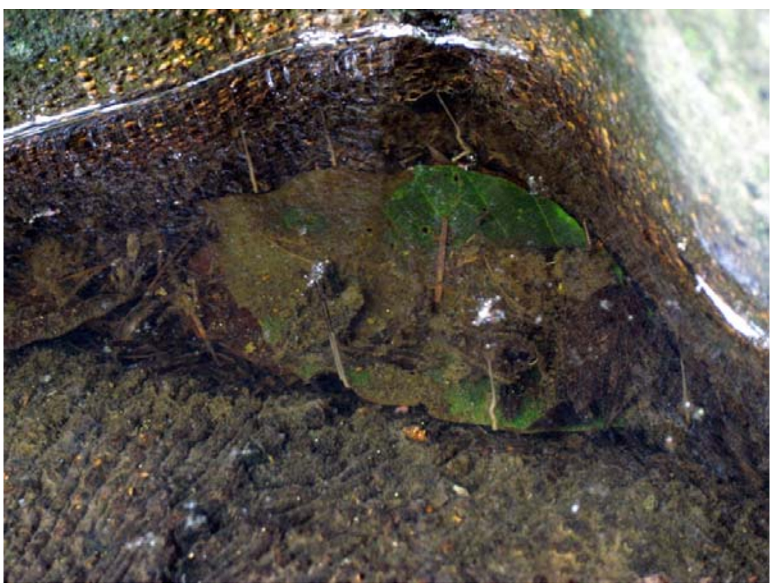

Figure S4-4: In the upper tree hole, the syrphid *Myathropa florea* was sometimes very abundant. Typically, individuals of this species stay vertically in the water with their long posterior breathing tubes erect and reaching the surface of the water, as photographed on 27 June 2009. Photograph credit: Martin M. Gossner.

Supplement S5: Raw species data

The raw data on the species sampled is provided as an Excel table:

Gossner_SpeciesRAW.xlsx