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1 **Life cycle of *Harmonia axyridis* in central Europe**

2

3

4 **Abstract**

5 The development and reproduction, and maximum number of generations across seasons were  
6 ascertained for populations of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in  
7 Central Europe. Number of generations was calculated using the thermal characteristics of  
8 development and summation of daily average air temperatures, in 2007–2016. This species  
9 can produce 2–3 generations per year, however, there is insufficient time for it to achieve its  
10 full reproductive potential. Delayed emigration from hibernacula (late April) shortened the  
11 period of seasonal activity by 1–6%. Breeding occurred from May to mid September.  
12 Oviposition continued into late summer, which left insufficient time for the late-hatching  
13 immatures to complete their development before the onset of winter. A high mobility,  
14 opportunistic use of prey patches and weak tendency to enter dormancy provide the potential  
15 for population increase by extending the period of development of immatures at the risk of  
16 failure if the cold period comes early.

17

18 **Key words**

19 Coccinellidae; development; generation; temperature; voltinism

20

21

## 22 **Introduction**

23 *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is an invasive non-native species that  
24 arrived in the Czech Republic in 2006 (Sprynar, 2008). In less than three years, from 2007–  
25 2009, it became a dominant ladybird species principally occurring on trees but frequently also  
26 on wild herbaceous plants and crops. *Harmonia axyridis* is not only an efficient predator of  
27 aphids (Roy et al. 2016) and other phytophagous insect pests (Qureshi and Stansly 2009) it is  
28 also an intraguild predator of native aphidophagous coccinellids (Ingels et al. 2013). As a  
29 consequence *H. axyridis* is considered to disrupt assemblages of native aphidophagous  
30 species (Pell et al. 2008). *Harmonia axyridis* is further accused of adversely affecting wine  
31 production and large aggregations in buildings of inducing allergic reactions and other  
32 medical conditions (Chakir et al. 2016). Consequently, the biology of *H. axyridis* has been  
33 intensively studied. However, despite this interest much of the basic life history data for this  
34 species has not been established with sufficient reliability.

35 Factors determining the number of generations per year (voltinism) are many and,  
36 despite intensive studies, still incompletely analyzed (Hodek 2012). This is also true of  
37 populations of *H. axyridis* in areas it has recently colonized. As in other coccinellids, its  
38 voltinism is specific for a particular geographic area with its specific climatic and biotic  
39 conditions (Roy et al. 2016). The temporal window for reproduction opens with leaving  
40 hibernacula in spring and closes with a return migration in autumn. The maximum number of  
41 generations they can complete during the vegetative period is controlled by abiotic conditions,  
42 temperature, which determines the pace of life and photoperiod, which governs the initiation  
43 of dormancy and may curtail the length of the period potentially available for reproduction.  
44 Further intrinsic (oviposition structure) and extrinsic factors (prey availability, mobility) limit  
45 the potential for reproduction. Assessments of voltinism in particular areas (Roy et al. 2016)

46 differ as they were established by studies carried out in different years and using different  
47 methods of sampling and data analysis (Brown et al. 2008).

48 While aspects of migration (Nalepa et al. 2005) and overwintering (Raak-van den Berg  
49 et al. 2012) are well studied, the factors determining the time they leave and return to  
50 hibernacula remain to be studied with respect to local conditions. There are many studies on  
51 the factors determining reproduction within the „window“ of the breeding season. In this  
52 respect there are studies on species' thermal characteristics (Schanderl et al. 1985; LaMana  
53 and Miller 1998), temperature effects on development and reproduction (Zazycki et al. 2015,  
54 Guedes et al. 2016) and variation in development rate of native and invasive populations  
55 (Raak-van den Berg et al. 2017). Under natural conditions duration of development varies  
56 with the course of a season, along with changing temperature. Photoperiod influences  
57 duration of development and ovarian maturation (Raak-van den Berg 2013). Invasive  
58 populations are less sensitive to photoperiodic dormancy induction in the autumn  
59 (Ovchinnikova et al. 2016). Effect of food availability and quality on development and  
60 oviposition in coccinellids is obvious (Hodek and Evans 2012) and can determine the length  
61 of the larval period (Phoofolo et al. 2009) and oviposition in adults (Hokusima and Ohwaki  
62 1972). As *H. axyridis* is attracted to both the odour of host plants and aphid prey (Rondoni et  
63 al. 2017) and can fly between host plants (Osawa 2000) it is capable of searching many host  
64 plants, aggregating at the most suitable sites and continuing reproduction for most of the  
65 season. The spatial and temporal redistribution of local populations in the landscape and its  
66 change during the course of a season, however, needs more study.

67 A synthesis of the following different kinds of information is needed for determining the  
68 voltinism of *H. axyridis*: the maximum number of generations based on local meteorological  
69 data, thermal characteristics of the development and oviposition of *H. axyridis* and the factors  
70 that set limits to the theoretically possible extent of their reproductive activity. We predict that

71 the seasonal course of the life cycle of *H. axyridis* is (i) driven by thermal time (rate of  
72 summing of effective temperatures above lower development threshold), (ii) constrained by  
73 spring emigration from and autumn migration to hibernacula and (iii) limited by availability  
74 of prey necessary for reproduction. In this study we therefore first calculate the potential  
75 course of seasonal development as determined by ambient temperature and species thermal  
76 norms. Then we establish times of migration from and to hibernacula. Third we establish the  
77 temporal patterns in oviposition.

78

## 79 **Material and Methods**

### 80 **Calculating potential life cycle**

81 Thermal constants, lower development threshold  $LDT = 10.6\text{ }^{\circ}\text{C}$  and sum of effective  
82 temperatures  $SET = 256$  day degrees (dd) were used for calculating duration of the pre adult  
83 developmental period (LaMana and Miller 1998; Schanderl et al. 1985). Assuming the  
84 developmental rate is isomorphic (Jarosik et al. 2002), which is typical for coccinellids  
85 (Hodek and Honek 1996; p. 63), the same lower developmental threshold  $LDT = 10.6\text{ }^{\circ}\text{C}$  was  
86 used to calculate the duration of the teneral and oviposition periods. Sum of effective  
87 temperatures were recalculated from data published by Guedes et al. (2016) who provide data  
88 on the teneral and oviposition periods of individuals of an invasive population of *H. axyridis*  
89 collected in Brazil and reared at  $25\text{ }^{\circ}\text{C}$  and fed *Brevicoryne brassicae* (L.). For the calculation  
90 we used a transformation coefficient calculated as  $[\text{SET for development based on the data of}$   
91  $\text{Schanderl et al. (1985) and LaMana and Miller (1998)}] / [\text{SET for development based on the}$   
92  $\text{data of Guedes et al. (2016)}] = 256/325 = 0.790$ . By multiplying Guedes et al. (2016) data by  
93 this factor we get a  $SET = 69$  dd for completing the teneral period (interval between adult  
94 moult and start of oviposition) and  $SET = 895$  dd for the oviposition period. Sum of effective

95 temperatures for the period from the beginning of development until beginning of oviposition  
96 is then SET = 325 dd (256 dd for development + 69dd for the teneral period).

97       The potential number of generations was calculated using average daily air temperatures  
98 measured at the meteorological station of the Crop Research Institute at Prague-Ruzyne  
99 (50.0852N, 14.2986E, 338 m a.s.l., <http://www.vurv.cz/meteo/default.asp>). Actual thermal  
100 time for individuals may differ slightly from the sum of temperatures based on summation of  
101 daily averages because temperature minima may fall below LDT (c. 30% of days with  
102 average temperature above LDT) and maximum temperatures may rise above the 30°C, the  
103 upper developmental threshold (10% of days). Precise estimation of thermal time was  
104 impracticable because actual body temperatures of individuals determined by microclimate  
105 and thermoregulation were not available. The calculations were made for 2007–2016, the ten  
106 years when *H. axyridis* became established in the Czech Republic. Each year, the daily  
107 average temperatures above LDT = 10.6 °C threshold (TD) were summed starting from  
108 January 1, an arbitrary date based on diapause being terminated in December (Raak-van den  
109 Berg et al. 2013). As average temperatures in December were invariably below LDT,  
110 temperature summation of the SET for post-diapause development may have started from  
111 January 1 and the TD summed until the date when a sum of TD 69 dd had accumulated, which  
112 is the duration of the teneral period. This was also assumed necessary for the maturation of  
113 eggs during the pre-oviposition period of overwintered post-diapause females. Thermal  
114 requirements for both these life periods are likely to be similar (Hodek and Honek, 1996,  
115 p.79) provided that food conditions are optimum (Hukusima and Kamei, 1970). This day was  
116 considered to be the date on which the overwintered females, the parental (P) generation,  
117 started laying eggs and is used as the starting point for calculating the potential number of  
118 generations that may develop in the following season. Starting from this date TD were  
119 summed until 256 dd (indicating date of termination of development of the first generation

120 F1) and 325 dd (date of start of oviposition of F1 generation) had accumulated. Then  
121 following from this date the temperatures TD were summed again until 256 dd (terminating  
122 development of second generation F2) and 325 dd (start of oviposition of F2) had  
123 accumulated, and then repeated again to determine the date when development was finished  
124 and the oviposition of the third generation F3 started. The days necessary for the particular  
125 sums of dds to be accumulated indicated the earliest dates on which adults of the first (F1),  
126 second (F2) and third (F3) generations could have completed their development and started  
127 ovipositing.

128 For each of the generations P to F3 we calculated the percentage of the oviposition  
129 period (895 dd) that was realized in the course of a vegetative season before the onset of  
130 winter. For the P generation the summation of TD started from the day of the start of  
131 oviposition of overwintered females until the date when 895 dd were accumulated, which was  
132 considered to be the date on which the P generation ceased ovipositing. For the F1 generation  
133 summation of TD started from the date of the start of oviposition of the F1 generation and  
134 continued until the end of the vegetative season (the date when autumn temperatures  
135 decreased below 10.6 °C and further development was not possible). Each year this date came  
136 earlier than that necessary for the accumulation of the 895 dd needed for completing  
137 oviposition. The proportion of the oviposition period realized until end of vegetative season  
138 was calculated. The same process was repeated for F2 and F3 generations.

139

#### 140 **Leaving hibernacula in spring**

141 Leaving artificial hibernacula was recorded in 2015 and 2016. Experimental adults were  
142 collected during the mass autumn migration and stored in plastic bottles at + 5 °C until the  
143 beginning of the experiments. On 10 November 2014 and 8 November 2015 they were put  
144 into 40 x 20 x 8 cm paper boxes perforated with holes enabling free access of beetles and



145 filled with egg cartons, which provided hiding places. The boxes wrapped in nylon fabric  
146 bags and black plastic bags with small ventilation holes were put into an unheated shelter  
147 whose conditions may approach that of the hibernacula this species frequently uses in  
148 buildings. Each bag was connected via a transparent tube to a transparent plastic vial. In the  
149 spring, adults leaving the artificial hibernacula in paper boxes passed through the tube into the  
150 sampling vials where they were counted and removed at 3–4 day intervals, until all  
151 individuals had left.

152

### 153 **Reproduction in the open**

154 In 2015 and 2016, the percentage of ovipositing females and size of egg batches was  
155 established at 18 sites in the west of the Czech Republic situated in an 11 x 7 km area centred  
156 at 50.105N and 14.264E. The beetles were sampled on trees (*Acer platanoides* L., *A.*  
157 *pseudoplatanus* L., *A. campestre* L., *Betula pendula* Roth, *Prunus insititia* L., *Tilia cordata*  
158 Mill., *Tilia platyphyllos* Scop.), herbaceous plants (*Tripleurospermum inodorum* (L.) Schultz-  
159 Bip., *Urtica dioica* L.) and crops (small grain cereals *Avena sativa* L., *Hordeum vulgare* L.,  
160 *Triticum aestivum* L.), using a standard sweepnet (35 cm diam., 140 cm handle). Sampling  
161 sessions, collecting adults at particular sites and on particular host plants were repeated at 7–  
162 14 d intervals.

163 The course of oviposition was investigated using a method used previously for native  
164 species (Honek et al. 2007). Individual females were each put in an Eppendorf tube perforated  
165 for ventilation and kept for 48 h at 25 °C under a natural photoperiod. The number of eggs  
166 was counted and eggs were removed (to prevent cannibalism) at 2-6 h intervals. In most cases  
167 eggs were deposited in one batch, within the first day. The percentage of ovipositing females  
168 in a sample originating from a particular session and number of eggs deposited per female  
169 were recorded.

170 Proportion of females that oviposited was compared among months using GLM with  
171 binomial errors and logit link (GLM-b). The number of eggs laid (batch size) was compared  
172 among months using GLM with Poisson errors and log link (GLM-p). Quasibinomial and  
173 quasipoisson setting, respectively, was used due to overdispersion. Post hoc comparisons  
174 among months were made using contrasts.

175

## 176 **Autumn migration**

177 The dates of autumn mass flights to hibernacula were established by daily observations of  
178 migration activity made from September 20 until November 10. Landing of flying adults was  
179 checked on a 15 m section of the south-facing light coloured wall of the building of the Crop  
180 Research Institute at Prague-Ruzyne, every day at 13:00, for 15 minutes. The wall section was  
181 inspected visually and the coccinellids that landed were counted and removed. To determine  
182 the days on which a massive flight occurred a threshold flight activity of  $\geq 3$  individuals  
183 landing per 15 min. session was set. In fact, the numbers of beetles that landed was either  
184 much higher on days when they were migrating, or zero.

185

## 186 **Results**

### 187 **Potential life cycle**

188 In 2007–2016, the maximum potential number of generations that could be completed given  
189 the local thermal environment was three per year (Table 1). It was predicted that the  
190 overwintered population (probably a mixture of all generations that developed the previous  
191 year) started ovipositing in early May (average May  $7 \pm 2.9$  d) and continued ovipositing until  
192 late August – early November (average September  $8 \pm 6.7$  d). Adults of the 1st generation  
193 (F1) originating from the first egg batches laid by overwintered females may appear in late-  
194 June (average June  $23 \pm 2.2$  d) and they started ovipositing seven days later (June  $30 \pm 2.1$  d).

195 Adults of the 2nd generation (F2) were predicted to appear in late July (July 29 ± 1.2 d) and to  
196 start ovipositing one week later (Aug 4 ± 1.5 d). The first adults of the 3rd generation (F3)  
197 may start emerging in late August – September (September 5 ± 3.1 d) and ovipositing ten  
198 days later (September 15 ± 4.1 d). Not even the 1st generation has sufficient time to complete  
199 oviposition, with only 79 ± 2.6% of the potential oviposition period realized before the end of  
200 the vegetative season. Females of the second generation may realize only 43 ± 2.5% of their  
201 potential oviposition period. The 3rd generation was predicted to have oviposited in only  
202 seven out of the ten years considered and the females then may only have realized 15 ± 3.6%  
203 of their potential oviposition capacity. Towards the end of the vegetative season, development  
204 may be completed before the onset of winter only if eggs are laid earlier than 256 dd before  
205 the average temperature falls below 10.6 °C. In particular years this critical date occurred  
206 between Aug 12 and Aug 28, and on average on August 19 ± 2.0 d. The individuals that  
207 hatched from eggs deposited later are unlikely to survive.

208

### 209 **Seasonal activity of adults**

210 The time schedule indicates that the maximum number of generations that can develop during  
211 the course of a year is limited by biotic factors. The first is the length of time each year during  
212 which the adults are active, which is determined by when they leave hibernacula in spring and  
213 migrate to hibernacula in autumn. They began leaving artificial hibernacula (Figure 1) in mid-  
214 March and continued for c. 60 days until early May. April 20, the median date for emigration  
215 of 50% of the overwintering individuals in 2015 (April 16) and 2016 (April 23) was used as a  
216 corrected time for the start of the temperature summation. In all years this date was later than  
217 the beginning of the period with average temperatures exceeding 10.6 °C. Moving the start of  
218 temperature summation to April 21 postponed the start of thermal time summation in  
219 particular years by 8.4–65.6 dd, i.e. by 0.5–5.8% of the total annual temperature sum

220 available in these years. In particular years this later start of temperature summation  
221 postponed the predicted time of oviposition of the overwintered generation by 1–20 days, the  
222 time of emergence of adults of the F1 generation by 1–11 d, F2 generation by 0–5 d and F3  
223 generation by 0–5 d.

224 In autumn a large percentage of the adults cease foraging on plants and migrate to  
225 hibernacula. In 2009–2016 mass flights occurred between October 2 and October 19, and  
226 smaller flights were recorded between October 30 and November 3 (Figure 2). The days on  
227 which mass flight occurred were very sunny, the temperature was above the average recorded  
228 in the previous few days (daily maxima  $19.2 \pm 0.83$  °C) and there was little or no wind (0.0–  
229 0.1 m s<sup>-1</sup>). The autumn migrations occurred before the end of the period when average daily  
230 air temperatures exceed 10.6 °C. In particular years this period finished on October 8 to  
231 November 11 (average October  $23 \pm 4.9$  d).

232

### 233 **Oviposition**

234 The seasonal course of oviposition was recorded because it may modify seasonal course of  
235 activity as predicted by the temperature summation. In 2016, ovipositing females were  
236 recorded between May 9 (samples from *P. insititia* colonized by *Brachycaudus helichrysi*  
237 Kaltenbach) and September 14 (samples from *T. cordata* colonized by *Eucallipterus tiliae*  
238 (L.)). Proportion of ovipositing females in samples collected from natural populations on trees  
239 decreased significantly (GLM-b,  $F_{4,58} = 7.7$ ,  $P < 0.0001$ ) throughout the vegetative season  
240 from 49% in May and 57% in June to 20% in August and 19% in September (Figure 3). Batch  
241 size varied between 1–60 eggs (mean  $19.8 \pm 0.81$  eggs,  $n = 238$  batches, Figure 4) and was  
242 not significantly different each month throughout the season (GLM-p,  $F_{4,233} = 0.18$ ,  $P = 0.95$ ).  
243 Average size of the batches of eggs laid in the field ( $26 \pm 3.6$  eggs,  $n = 9$ , on *P. insititia*) was  
244 greater than that laid by the females in the samples collected in the field and then kept

245 individually in tubes in a laboratory. In 2015, ovipositing females were recorded on *Acer*,  
246 *Betula* and *Tilia*. Oviposition occurred only between May 5–July 13 (although sampling  
247 continued until mid-October). Mean batch size was  $21.7 \pm 3.83$  eggs (range 1–57 eggs,  $n = 77$   
248 batches), which is similar to that recorded in 2016.

249

## 250 **Discussion**

251 In this paper we determine when in the year *H axyridis* reproduces and develops, and how the  
252 potential number of generations it has is determined by local thermal conditions. We specify  
253 some of the factors that can modify this potential scenario: time of leaving and entering  
254 hibernacula and seasonal reproduction patterns. Calculations presented are of the fastest path  
255 for development: a sequence of life histories of the earliest developed individuals in each  
256 generation. This is an ideal sequence of successive generations, which may be modified by  
257 abiotic (microclimate, photoperiod) and biotic (thermoregulation, food) factors. The  
258 relationship between the predicted and actual species life cycle thus needs consideration.

259

### 260 **Thermal limits of the life cycle**

261 The temporal course of an insect's life history is shaped by temperature, which determines  
262 their pace of life (Dixon 2015; Trudgill et al. 2005). Our calculations (Table 1) using local  
263 temperature data predicts three generations per year, except in 2010 when only two  
264 generations were predicted. In seven of the years considered the thermal conditions remained  
265 suitable for even a 3rd generation to start reproducing. However, only overwintered females  
266 (P generation) have sufficient time to realize their full reproductive potential, on average  
267 ceasing to reproduce as late as September. None of the generations born during the course of  
268 a year (F1 – F3) achieved their full reproductive potential. Even for the F1 generation the  
269 vegetative season terminated earlier than the first born adults of this generation, which were

270 predicted to complete their development in late June, leaving only 80% of the thermal time  
271 needed to realize their reproductive potential and even less thermal time for the second (42%)  
272 and third (10%) generations. These predictions based on laboratory established thermal  
273 requirements and standard meteorological measurements of temperature may be affected by  
274 two further factors.

275 First, active behavioural thermoregulation may increase body temperature above air  
276 temperature. Selection of warm places available within their habitats is an important factor  
277 determining the distribution of coccinellids (Honek 1985). This thermoregulatory behaviour  
278 is typical of several species of coccinellids including *Coccinella septempunctata* L. (Honek  
279 1982). Adults of *H. axyridis* also aggregate in the more sunny sections of tree crowns, where  
280 they make full use of high leaf temperatures and bask in sunshine. Active behavioural  
281 thermoregulation may be an efficient means of increasing the rate of thermal summation in  
282 adults, particularly in cool periods in spring and autumn (Michie et al. 2010). Consequently  
283 the reproductive potential of adults may be higher than predicted using meteorological data.  
284 Behavioural thermoregulation is used by adults of *Adalia bipunctata* (L.) to increase their  
285 body temperature several degrees above ambient temperature, depending on colour form and  
286 body size (Stewart and Dixon 1989). The different colour morphs of *H. axyridis* differ in their  
287 thermoregulatory properties, which is reflected in the geographical (Komai et al. 1950; Osawa  
288 and Nishida 1992) and seasonal variation (Dobzhansky 1924a; Komai 1956), and frequency  
289 of melanic (*conspicua* and *spectabilis*) and pale (*succinea*) morphs in local populations.  
290 Extent of melanism in the *succinea* morph is moreover affected by the number of spots on its  
291 elytra (genetically determined) and variation in their size, which increases with decreasing  
292 development temperature and may affect adult thermoregulation (Michie et al. 2010; Knapp  
293 and Nedved 2013). However, mating activity of melanic and pale morphs on cold mornings  
294 and warm afternoons do not differ (Awad et al. 2015). *Succinea* and *spectabilis* morphs also

295 differ in their demographic parameters, intrinsic rate and finite rate of population increase,  
296 which are greater in the former morph (Zeki et al. 2015). As in *C. septempunctata* (Ohashi et  
297 al. 2005), thermoregulatory behaviour might also affect the selection of a pupation site. In the  
298 Czech Republic *H. axyridis* selects warm places for pupation, which may increase its survival  
299 and enable it to complete its development in cold autumns. Pupae of all morphs also display  
300 thermal melanism, which decreases differences in body temperature throughout the season.  
301 By contrast, development of eggs is less likely be affected by thermoregulation. In  
302 coccinellids, oviposition sites are selected with respect to availability of prey for the progeny  
303 and protecting eggs from intraguild predation (Seagraves 2009). Selecting oviposition sites  
304 based on these environmental characteristics leaves less freedom for selecting a more  
305 favourable thermal environment. Larvae spend most of their time foraging for prey. In cold  
306 weather coccinellid larvae often assemble on the insulated tops of leaves and aphids also  
307 move onto upper leaf surface to benefit from the warming rays of the sun (A.F.G. Dixon,  
308 unpublished). However, sections of crown where they gather are insulated for only a part of  
309 day and the larvae cannot keep pace with changing insolation as fast as adults, which move by  
310 flight. Larvae are therefore limited in making full use of active thermoregulation. The effect  
311 of thermoregulation on pre-adult development is thus probably smaller than on adult  
312 reproduction.

313         Second, there may be problems with the data on the thermal reactions of *H. axyridis*  
314 used in this study. The 895 dd available for oviposition calculated from Guedes et al. (2016)  
315 is the maximum estimate of the length of the oviposition period. Using the same method of  
316 recalculation and the results of Awad et al. (2013) and Zazycki et al. (2015) provided similar  
317 results (873 dd, 799 dd and 1051 dd, respectively) while the results of another study by  
318 Lanzoni et al. (2004) resulted in a much shorter oviposition period (183 dd). The thermal  
319 characteristics were calculated using data from experiments using strains from different

320 geographic regions. Thermal requirements of populations native to particular geographic areas  
321 may be adapted to local conditions as has been demonstrated for several insect taxa (Kiritani  
322 2012), such as Homoptera (Umeya and Yamada 1973), Heteroptera (Balashov and Kipyatkov  
323 2008), Coleoptera (Lopatina et al. 2011) and Hymenoptera (Kipyatkov and Lopatina 2015).  
324 Differences among geographic populations are mostly small but nevertheless ecologically  
325 important. Whether this differentiation in thermal requirements exist in native populations of  
326 *H. axyridis*, and the extent to which it is already present in very recently spreading invasive  
327 populations of this species is difficult to estimate. Potentially, recalculating thermal constants  
328 using data of local populations may improve the prediction of the seasonal cycle of *H.*  
329 *axyridis*.

330

### 331 **Biotic modification of life cycle**

332 There are several biotic factors that decrease the thermal time available for the development  
333 of *H. axyridis*. The annual life cycle starts when adults leave hibernacula. Although *H.*  
334 *axyridis* may overwinter on conifers (Holecova et al. in prep) and in hilltop hibernacula used  
335 by *C. septempunctata* and *Hippodamia variegata* Goeze (unpublished authors observation)  
336 there is good evidence of massive overwintering in buildings (Lucas et al. 2007; reports from  
337 the public). Our experiment using artificial hibernacula thus provides the timing of the spring  
338 migration typical for a large part of the *H. axyridis* population, which extends over two  
339 months. As for native species of coccinellid (Honek 1986) early emerging individuals may  
340 feed on alternative prey. The major peak of emigration in late April coincides with leaf  
341 expansion of their preferred tree hosts, *Tilia* spp. and *Acer* spp. and development of aphid  
342 populations on primary hosts (Leather and Walters 1984).

343 Low food specificity is typical (Weber and Lundgren 2009) and beneficial (Hodek and  
344 Evans 2012) for predaceous coccinellids, including *H. axyridis* (Lucas et al. 2004). Eating



345 alternative prey, Homoptera, psyllids, aleyrodids, coccids, Heteroptera, Thysanoptera,  
346 Coleoptera, Lepidoptera and mites, or a mixed diet do not only enable adults to survive early  
347 and late in the vegetative season but supports development during the breeding period (Howe  
348 et al. 2016; Lucas et al. 2002). However, despite wide polyphagy, presence of aphids is the  
349 main factor determining the temporal distribution of *H. axyridis*. Prey abundance limits the  
350 percentage of individuals that realize their reproductive potential in particular generations and  
351 the length of the oviposition period at different sites. This limitation is substantial and may  
352 seriously distort the patterns of activity calculated using thermal data.

353         The average percentage of ovipositing females in populations (maximum c. 60% in  
354 June, Figure 3) was lower than in populations of *C. septempunctata* and *Propylea*  
355 *quatuordecimpunctata*, where it exceeds 80% (Honek et al. 2008). We propose that a high  
356 percentage of non-ovipositing females assures a more flexible strategy in which females may  
357 continue to move among host plant patches in search of other sites favourable for  
358 reproduction. The number of eggs ready to be laid (clutch size) is smaller in *H. axyridis* (~20  
359 eggs) than in similarly sized *C. septempunctata* (~36 eggs) (Honek et al. 2007). Similar  
360 differences are reported in which the mean batch size for *H. axyridis* is 23–31 eggs  
361 (Dobzhansky 1924b; Takahashi 1987) and for *C. septempunctata* 33–50 eggs (Stewart et al.  
362 1991; Takahashi 1987). This variation in batch size may reflect differences in the number of  
363 ovarioles, which is smaller in *H. axyridis* (62–70, mean  $65 \pm 1.3$ ) than in *C. septempunctata*  
364 (74–149, mean  $109 \pm 9.6$ ) (Nedved and Honek 2012). The partition of egg load into many  
365 small batches enables a species to lay eggs on many more host plants, thus decreasing the risk  
366 of failure to develop that threatens populations in any particular patch (den Boer 1981).  
367 Together with high mobility the tendency to partition oviposition in this way may assist *H.*  
368 *axyridis* in locating and exploiting small or more inaccessible patches of prey (Osawa 2000).  
369 This opportunistic strategy may have enabled *H. axyridis* to become a conspicuous and

370 characteristic component of coccinellid communities in small patches of vegetation in urban  
371 areas (Honek et al. 2017).

372 In late summer females continued to oviposit until mid-September (Figure 3), far  
373 beyond the "critical date" for completing development before the onset of winter (August 19  
374  $\pm$  2.0d, Table 1) and near the ultimate date of oviposition established for native *H. axyridis*  
375 populations in Far East Russia (Telenga and Bogunova 1936). Despite their ability to  
376 thermoregulate some of these individuals are likely to be killed at the onset of cold weather.  
377 Development may occur also on days with low average temperatures if afternoon  
378 temperatures substantially exceed LDT, which is often the case in October. This may create  
379 conditions for completing development of some belated individuals even when forecasts  
380 using summation of average temperatures predict extinction. This poor adjustment of  
381 dormancy initiation in invasive populations of *H. axyridis* may be a consequence of a weak  
382 photoperiodic reaction and inability to respond to changing autumn conditions (Reznik et al.  
383 2015). In this study females continued to lay eggs under a short dormancy inducing  
384 photoperiod (13h light:11h dark or 14h light:10h dark including twilight on September 15). In  
385 order to prevent late oviposition and its fatal consequences dormancy should be induced on  
386 August 15, i.e. by a 14.5h light:9.5h dark photoperiod (or 15.1h. light:8.9h dark photoperiod  
387 including twilight).

388 Mass migration of *H. axyridis* to overwintering sites occurred in October. Each year  
389 there are few days suitable for migration. These days are characteristically warm, calm and  
390 sunny (Nalepa et al. 2005). These mass flights may be synchronized over large areas as on  
391 October 16, 2016 when massive flights were recorded simultaneously in Prague (50.1N,  
392 14.3E, Figure 2), in southern Moravia (49.2N, 16.6E, S. Pekár) and central Slovakia (48.6N,  
393 19.1E, Peter Zach, personal communication), that is at localities c. 400 km apart. Every year,

394 *H. axyridis* is recorded on trees even after the mass migration, where they remain until leaf  
395 fall.

396 Our method of calculating the maximum number of generations and their share in the  
397 total of the offspring produced in a given year provides important information for assessing  
398 the impact of *H. axyridis* on aphid and intraguild prey populations in a particular area. The  
399 results presented indicate the enormous reproductive potential of *H. axyridis*. If prey is  
400 available its breeding period is limited only by thermal conditions. Populations of *H. axyridis*  
401 continue to reproduce in to late summer regardless of the risk of some of the individuals  
402 perishing when there is an unusually early onset of the cold autumn period. We propose that  
403 the source of success of this strategy is, paradoxically, a (still) poor adaptation to local  
404 climatic conditions of *H. axyridis* that has only recently colonized the Czech Republic  
405 (Reznik et al. 2015). This “audacious and risky” strategy may have contributed to making *H.*  
406 *axyridis* an extremely successful invasive species.

407

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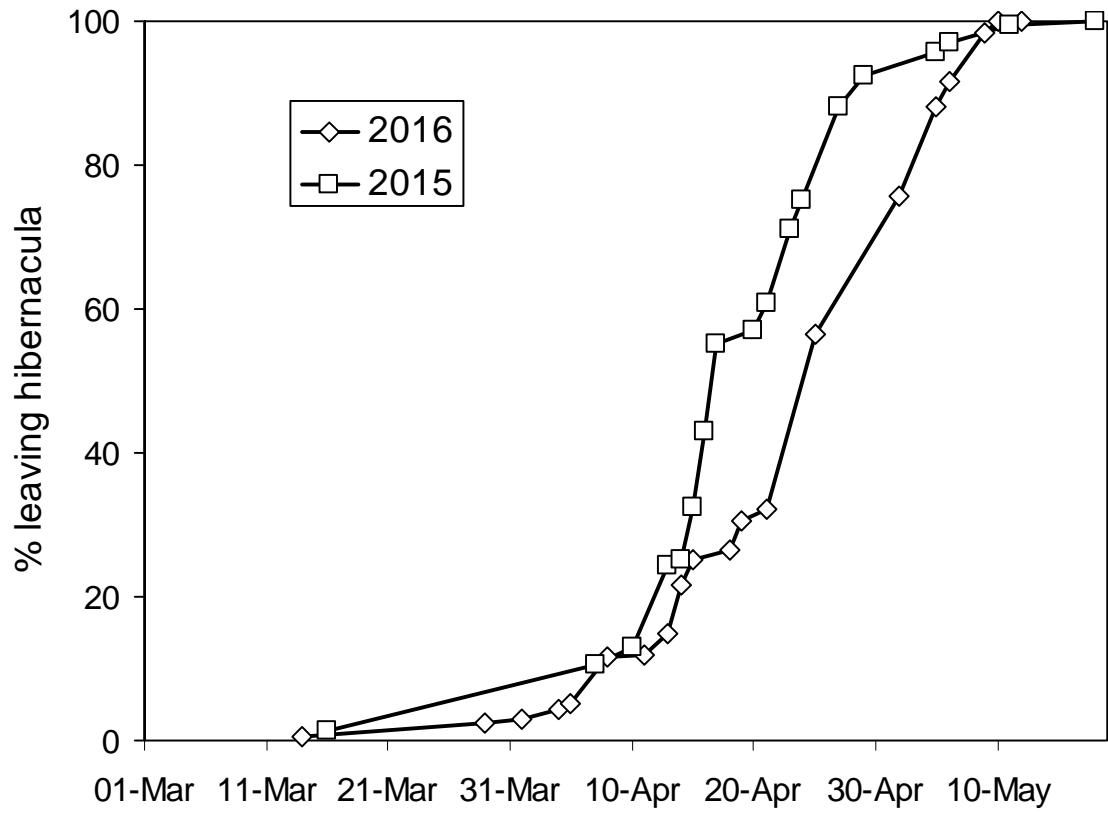
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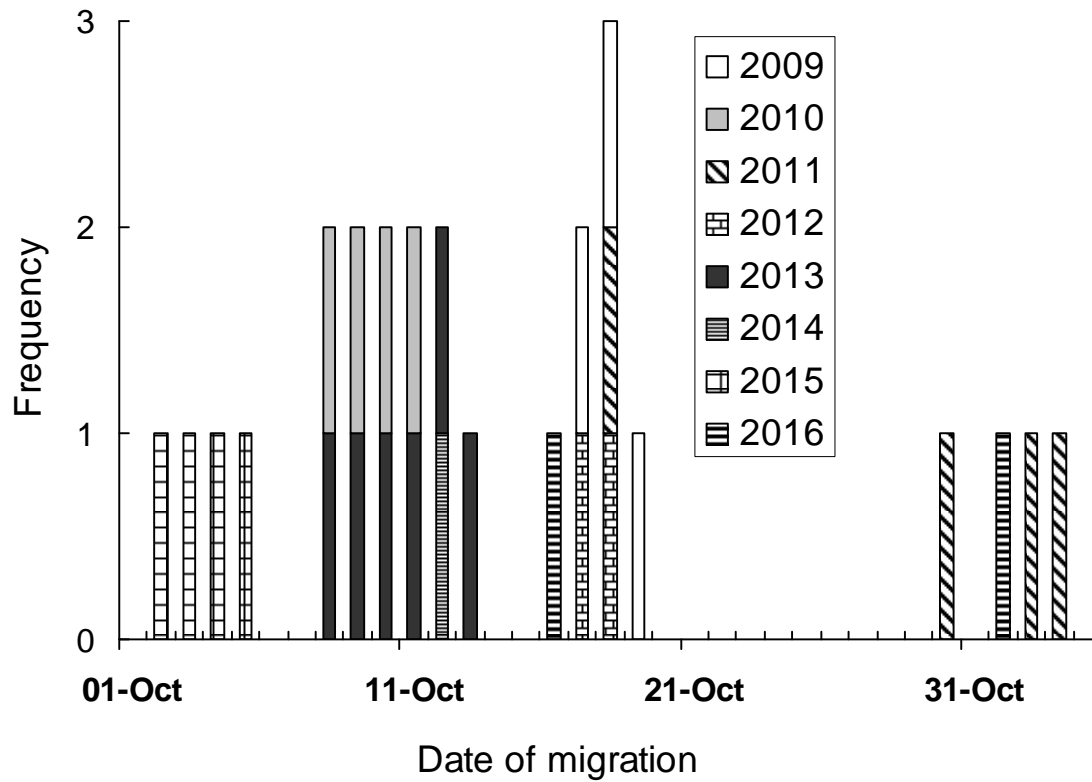
586 **Table 1** Timing of the development of successive generations of *H. axyridis* based on meteorological data collected from 2007 to 2016 at Praha-  
587 Ruzyne and its thermal requirements for development and oviposition (see Material and Methods). The date of the start (SOVIP) and termination  
588 (EOVIP) of oviposition is indicated for the overwintered generation (P). For each generation (F1 – F3) that developed during the course of the  
589 vegetative season the dates for the completion of pre-adult development (DEV), start of oviposition (SOVIP) and the thermal time available for  
590 oviposition until the end of the vegetative season (REALT, number of day degrees (dd) and percentage of the total period available for  
591 reproduction) are indicated . Critical date (DCRIT) is the date after which pre-adult development cannot be completed because the thermal time  
592 to the onset of winter is shorter than that needed for pre-adult development. Mean dates were calculated after converting dates of particular years  
593 to Julian days.

Year	Generation P		F1		F2		F3		OVIP	DCRIT					
	SOVIP	EOVIP	DEV	SOVIP	DEV	SOVIP	DEV	SOVIP			DEV				
					REALT dd	%			REALT dd	%					
2007	28-Apr	21-Aug	10-Jun	17-Jun	731.0	81.6	18-Jul	24-Jul	406.0	45.4	24-Aug	06-Sep	81.0	9.1	12-Aug
2008	17-May	06-Sep	24-Jun	01-Jul	639.0	71.4	01-Aug	07-Aug	309.0	34.5	09-Sep				13-Aug
2009	21-Apr	02-Sep	24-Jun	02-Jul	753.0	84.1	01-Aug	07-Aug	424.0	47.4	05-Sep	18-Sep	94.0	10.5	26-Aug
2010	22-May	04-Nov	01-Jul	08-Jul	575.0	64.2	01-Aug	10-Aug	246.0	27.5					12-Aug
2011	06-May	06-Sep	15-Jun	25-Jun	737.0	82.3	31-Jul	08-Aug	409.0	45.7	09-Sep	21-Sep	82.0	9.2	25-Aug
2012	02-May	26-Aug	19-Jun	27-Jun	736.0	82.2	26-Jul	01-Aug	423.0	47.6	27-Aug	08-Sep	95.0	9.5	20-Aug
2013	07-May	14-Sep	01-Jul	08-Jul	627.0	70.1	01-Aug	06-Aug	300.0	33.5	23-Sep				12-Aug
2014	06-May	14-Sep	24-Jun	05-Jul	688.0	76.9	30-Jul	06-Aug	359.0	40.1	17-Sep	10-Oct	32.0	3.6	21-Aug
2015	12-May	25-Aug	01-Jul	06-Jul	795.0	88.8	01-Aug	06-Aug	461.0	51.5	28-Aug	02-Sep	136.0	15.2	23-Aug
2016	14-May	01-Sep	23-Jun	30-Jun	790.0	88.3	27-Jul	04-Aug	454.0	50.7	04-Sep	11-Sep	123.0	13.7	28-Aug
Mean	07-May	08-Sep	23-Jun	30-Jun	707.1	79.0	29-Jul	04-Aug	379.1	42.4	05-Sep	15-Sep	91.9	10.1	19-Aug
SE	2.9d	6.7d	2.2d	2.1d	23.1	2.6	1.4d	1.5d	22.9	2.6	3.1d	4.1d	10.6	1.2	2.0d

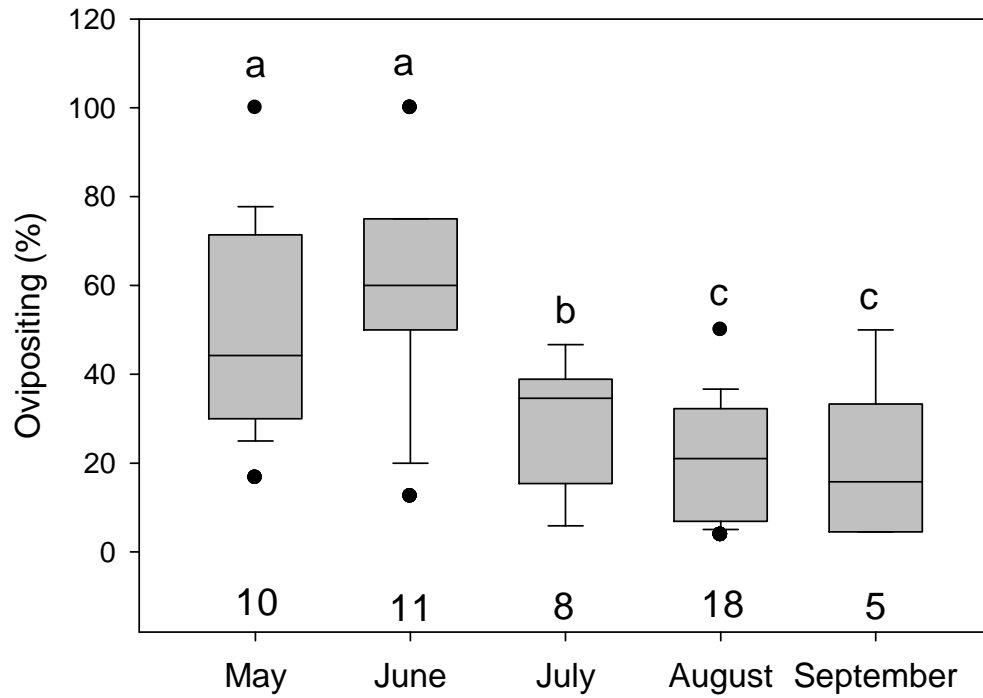
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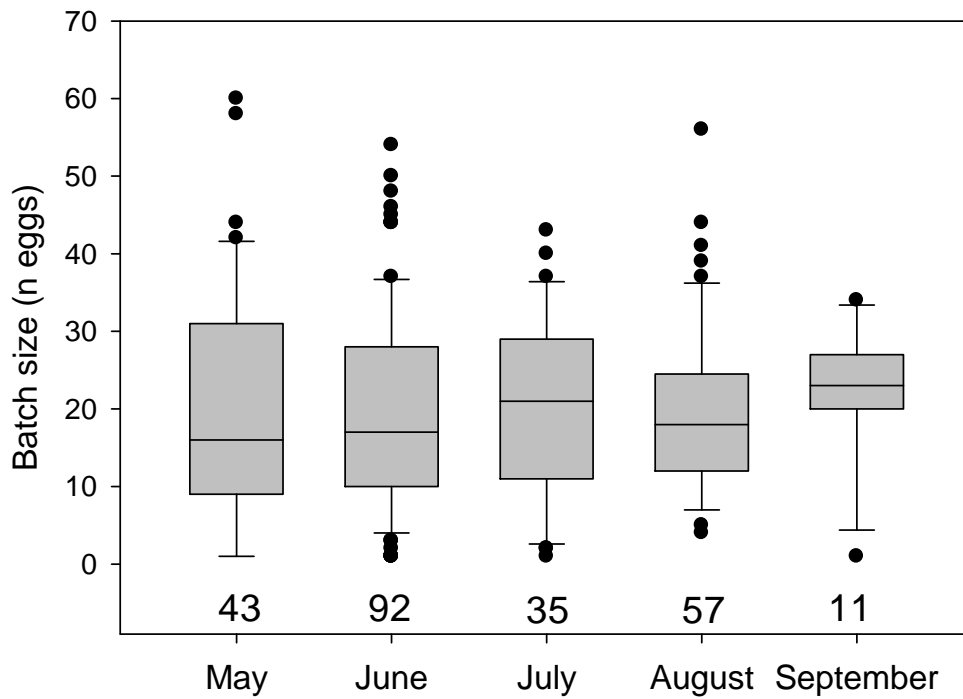
**Figure 1** Cumulative percentage of *H. axyridis* individuals leaving an artificial overwintering site in 2015 (n = 238 individuals) and 2016 (n = 771).



**Figure 2** Frequency at which autumn migration of *H. axyridis* to hibernacula was recorded on particular days, at Prague-Ruzyně over the period 2009–2016. For each year dates on which migration was recorded is indicated by a column of the same size and columns are stacked vertically if flight activity occurred on the same date in different years.



**Figure 3** Percentage of females of *H. axyridis* in populations (number of populations is indicated by the number at the bottom) that oviposited. Boxes topped by different letters differ significantly at  $P < 0.05$ . Differences compared using contrasts following GLM with binomial errors. Boxes: median and 25% and 75% percentiles, whiskers are 1.5 times the interquartile range. Points outside whiskers are considered as outliers.



**Figure 4** The sizes of the batches of eggs laid by females of *H. axyridis* (number of eggs laid by a female after collection from a natural population) in particular months in 2016 (number of batches is indicated by the number at the bottom). There was no significant differences in batch size among months. Differences compared using contrasts following GLM with Poisson errors. Boxes: median and 25% and 75% percentiles, whiskers are 1.5 times the interquartile range. Points outside whiskers are considered as outliers.