

ORIGINAL CONTRIBUTION

Effects of photoperiod and diet on diapause tendency, maturation and fecundity in *Harmonia axyridis* (Coleoptera: Coccinellidae)

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Abstract

The effects of photoperiodic conditions of larval development and adult maturation (L : D = 12 : 12 vs. 18 : 6) and different diets (sugar solution, frozen eggs of *Sitotroga cerealella*, different numbers of aphids *Myzus persicae*, and their combinations) on survival, reproductive maturation and fecundity of *Harmonia axyridis* were studied in laboratory conditions. The fundamental aim of the work was to distinguish between cue effect of diet (neurohormonal triggering of reproduction) and direct effect of diet (nutritional maintenance of reproduction). When adults were kept under short-day conditions, the proportion of ovipositing females decreased and the duration of the pre-oviposition period increased. Moreover, a strong reaction to the direction of changes in the day length was demonstrated: when larvae and pupae developed at long day and adults were transferred to short day, the proportion of ovipositing females was much lower than in individuals that were permanently kept under short-day conditions. The percentage of ovipositing females, the rate of their reproductive maturation and the average daily fecundity gradually increased in the following succession of diets: 'sugar + 5 aphids per day < sugar + eggs < sugar + eggs + 5 aphids per day < sugar + 100 aphids per day'. However, dissection showed that most of the non-laying females fed on these diets (particularly those kept under long-day conditions) have started reproductive maturation, while even first stages of oogenesis were not found in females fed on sugar solution alone. We conclude that cue effect of diet (reproductive activation) can be achieved almost independently of the number of prey consumed, while nutritional effects (the rate of reproductive maturation and fecundity) are sensitive both to the quality and quantity of food.

Introduction

Adult or reproductive diapause is a syndrome including arrested reproduction, low metabolic rate, accumulation of reserves, increased resistance and other physiological and behavioural adaptations that allow an insect to survive adverse conditions and to postpone reproduction for a more favourable period. In contrast to quiescence that is directly provoked by unfavourable conditions (e.g. extreme temperatures,

drought), facultative diapause is an anticipatory reaction induced by environmental tokens (most commonly day length). In reality, however, one and the same environmental factor can exert both direct and cue effects (Tauber et al. 1986; Danks 1987; Zaslavski 1988; Denlinger 2002).

Food is the most important of biotic environmental factors limiting insect development and reproduction. At the same time, shortage or low quality of food can promote induction of diapause, particularly among

phytophagous insects and insect predators (Danks 1987; Reznik 1991; Hodek and Honěk 1996; Horton et al. 1998; Musolin and Saulich 2012). Thus, the effect of diet on insect reproduction may consist of informational and nutritional components that can be separated only in experiments (e.g. Perry and Roitberg 2005; Reznik and Vaghina 2006). In addition, induction of diapause may depend on the interaction of trophic factor with photoperiod (Kono 1982; Horton et al. 1998; Alekseev and Lampert 2004; Musolin and Saulich 2012).

It has been repeatedly demonstrated that maturation and fecundity of females of predatory coccinellids markedly depends on the diet (Hodek and Honěk 1996; Evans 2000; Abdel-Salam and Abdel-Baky 2001; Soares et al. 2004, 2005; Osawa 2005; Berkvens et al. 2008a,b; Sighinolfi et al. 2008; Santos et al. 2009; Omkar et al. 2010; Agarwala and Bhowmik 2011; Stathas et al. 2011). All potential prey and other types of food can be separated in two groups: (i) 'essential' or 'nursery' foods that stimulate oogenesis and oviposition in females (and also support normal growth and development of the progeny) and (ii) 'alternative' or 'supplementary' foods that can only ensure relatively long-term survival of adult predators (Hodek and Honěk 1996; Dixon 2000). Moreover, in certain of the predatory lady beetles, reproductive activity can be triggered only by consumption of particular species of prey and diet is the main or even the only cue factor inducing reproductive diapause (Wipperfürth et al. 1987; Zaslavski et al. 1998; Dixon 2000; Evans and Gunther 2005; Reznik and Vaghina 2006). Thus, low-quality food may cause termination of oviposition by two different ways: directly (by deficiency of nutrients necessary for oogenesis) and indirectly (via neurohormonal regulation).

The object of our study, the multicoloured Asian lady beetle, *Harmonia axyridis* (Pall.), is a generalist predator (Hodek and Honěk 1996). This predaceous coccinellid was widely used for biological control of insect pests in field and in greenhouses, it has been imported to many countries, but unexpectedly *H. axyridis* turned to be able to invade natural ecosystems and is now considered to be a dangerous invasive alien species (Koch 2003; Koch and Galvan 2008; Roy and Wajnberg 2008; Soares et al. 2008).

Various aspects of *H. axyridis* biology have been studied by different authors. It has been demonstrated that in spite of the wide polyphagy of this predator, the rate of maturation and fecundity of females depend on their diet (Hodek and Honěk 1996; Abdel-Salam and Abdel-Baky 2001; Soares et al. 2004, 2005; Berkvens et al. 2008a,b; Sighinolfi et al. 2008;

Santos et al. 2009; Stathas et al. 2011). In addition, low-quality 'alternative' food (as well as the short day) may cause an increased tendency to reproductive diapause (Voronin 1965; Sakurai et al. 1988; Ongagna and Iperiti 1994; Hodek and Honěk 1996; Iperiti and Bertrand 2001; Osawa 2005; Berkvens et al. 2008a,b; Reznik and Vaghina 2011). However, in most of the above-cited studies, direct (nutritional) and cue (informational) effects of diet on reproduction were not clearly separated.

For example, Evans and Gunther (2005) have published very interesting data on the effects of different types of food on oviposition and fecundity of *H. axyridis*, but in this study, non-laying females have not been dissected and it remained unclear whether the oogenesis was completely terminated by the neurohormonal system or just slowed down by the deficiency of nutrients.

Berkvens et al. (2008b) have studied the effects of two prey species and day length on the pre-oviposition period, mean daily fecundity and the induction of diapause in different morphs and populations of *H. axyridis*. In particular, it was demonstrated that the photoperiodic effect in females fed on frozen eggs of *Ephestia kuehniella* Zeller was much stronger than that in females fed on the pea aphid *Acyrtosiphon pisum* (Harris). Moreover, feeding on eggs caused significantly longer pre-oviposition periods than feeding on aphids in all cases except for females of the laboratory population reared at a 16-h photoperiod. These data suggest that food and photoperiod interacted as diapause-inducing environmental cues, although only samples of females fed *E. kuehniella* eggs at a 12-h photoperiod were dissected to confirm the diapause. In the other work (Berkvens et al. 2008a), effects of two diets (eggs and pollen) and their combination were investigated, and it was demonstrated that in females from the field populations, the pre-oviposition period was shorter when reared on the mixed diet, but females have not been dissected and again it was not possible to segregate nutritional (slowing) and informational (termination) effects of diet on reproduction. It is known that not only female maturation but also the rate of pre-imaginal development of *H. axyridis* depends both on photoperiod and on diet. Our recent study (Reznik 2010) suggests that in this particular case, diet does not play the role of cue factor, but only directly influences larval development.

In the present study, we examined effects of photoperiodic conditions of larval development and adult maturation, different diets and their combinations on female survival, tendency to diapause, rate of maturation and fecundity of *H. axyridis*. The

fundamental aim of the work was to distinguish between cue effect of diet (neurohormonal triggering of reproduction) and direct effect of diet (nutritional maintenance of reproduction). In particular, we hypothesized that nutritional effects (changes in the rate of maturation and fecundity) would depend on both the quality and quantity of food and mixed diet would produce cumulative nutritional effect (summation of effects of different components). The cue effect of the diet (reproductive activation), on the contrary, would depend only on the quality of food, and the effect of the mixed diet would not be the sum of the effects of its components. In addition, the results of our study may be of practical use both in the selection of the optimal diet for laboratory rearing of *H. axyridis* and in the analysis of its population dynamics and seasonal cycles in natural conditions where ladybirds can feed on very different types of prey and other foods.

Materials and Methods

The experiment was conducted with a laboratory strain of *H. axyridis* originated from insects collected in the Kedrovaya Pad' nature reserve (43.1°N, 131.5°E, Khasanskiy region, Primorskiy territory of Russia). In spite of the low latitude, the climate of this region is cold (the average no-frost period is 185 days, the average temperature of January is -15°C) and *H. axyridis* can only overwinter as diapausing adults. Before the experiment, the strain was reared for more than 30 generations under constant conditions (25°C, L : D = 18 : 6) feeding on nymphs and adults of the green peach aphid, *Myzus persicae* (Sulz.) (Homoptera: Aphididae) reared on broad bean, *Vicia faba* L. seedlings (aphids are the natural food of *H. axyridis*). In spite of long-term rearing in laboratory, the pattern of the photoperiodic response of the studied strain is almost identical to that observed in natural conditions (Voronin 1965; Reznik and Vaghina 2011). This strain consisted predominantly (>95%) of the non-melanic (f. *succinea*) morph; the very few melanic adults were excluded from the experiment.

For the experiment, a cohort of the 1st instar larvae hatched during 12 h from eggs laid by 30 females of the laboratory strain was randomly distributed between two photoperiodic regimens (L : D = 16 : 8 or 12 : 12 in a thermostatic room at 20°C). The larvae were reared individually in plastic Petri dishes (90 × 15 mm), and food (aphids on bean seedlings) was provided daily in excess. Newly emerged adults were sexed, and the pairs were once again randomly distributed between the same two regimens (in few

cases, males randomly selected from the main laboratory strain were used). Thus, the experiment included four photoperiodic treatments (combinations of larval and adult regimens) hereafter referred as '12→12' (both larvae and adults were kept under short day L : D = 12 : 12), '18→18' (both larvae and adults were kept under long day L : D = 18 : 6), '12→18' (larvae were kept under short day and adults were kept under long day) and '18→12' (vice versa, larvae were kept under long day and adults were kept under short day).

In each photoperiodic treatment, pairs of adults were randomly assigned to five feeding regimens: (i) 'S' or 'sugar' (50% sugar solution provided in test tubes plugged with cotton plug plus a bean seedling); (ii) 'S + E' or 'sugar and eggs' (sugar solution provided as in the previous diet plus a bean seedling plus frozen eggs of the Angoumois grain moth *Sitotroga cerealella* Oliv. (Lepidoptera, Gelechiidae) provided daily in excess glued to hard paper); (iii) 'S + 5A' or 'sugar and five aphids' (sugar solution provided as in the previous diets plus 5 *M. persicae* adults provided daily on the bean seedling); (iv) 'S + E + 5A' or 'sugar and eggs and five aphids' (sugar solution plus five aphids plus *S. cerealella* eggs, all these foods being provided as in the previous diets); and (v) 'S + 100A' or 'sugar and 100 aphids' (sugar solution plus ca 100 aphids provided daily on the bean seedling). It has been demonstrated (Abdel-Salam and Abdel-Baky 2001; Berkvens et al. 2008b; Reznik 2010) that larvae and adults of *H. axyridis* can develop and reproduce when fed on frozen moth eggs, although aphids are their preferred natural food, while sugar solution can ensure female survival but not reproduction.

To determine the pre-oviposition period and fecundity, each Petri dish was checked daily for eggs. The duration of reproductive maturation of ovipositing females was recorded as the number of days from adult eclosion to the laying of the first egg. Sixty days after eclosion, the experiment was terminated and all survived non-ovipositing females were dissected to determine the state of their ovaries with the following rough scale: (i) 'diapause' (only germarium is present, follicles are absent), (ii) 'oogenesis' (stage I follicles, stage II follicles or even mature oocytes are present). The mean daily fecundity was calculated as the total number of eggs laid during the oviposition period divided by the duration of the oviposition period (with a very few exceptions, females that started to lay eggs continued to oviposit until the end of the experiment or until their death).

Survival, reproductive maturation (the stage of oogenesis at the end of the experiment) and fecundity

of 25–35 females were studied for each of 20 combinations of diet and photoperiodic regimen with a total of 559 females. Each female was considered as an experimental unit. The proportions of females that started to lay eggs, females that survived until the end of the experiment and those at different stages of oogenesis were compared using the chi-square test with the Mantel–Haenszel adjustment for the analysis of numerous contingency tables. The distribution of the duration of the pre-oviposition (reproductive maturation) period and mean daily fecundity of ovipositing females were not normal; these data were ranked and then analysed by ANOVA and the Tukey HSD test. All the calculations were made using SYSTAT.

Results

Survival

Analysis of the total data set by the chi-square test with the Mantel–Haenszel adjustment showed that the effect of the larval photoperiod (i.e. the photoperiod during the larval development) on adult mortality was not significant ($P > 0.4$) both with the adult photoperiod and with diet as a strata variable. The impact of the photoperiod that influenced adults (the larval photoperiod and diet were used as strata variables) was also not significant ($P > 0.5$). However, separate analysis of the data for females fed only with sugar solution (diet S) showed that when females whose pre-imaginal development occurred under

short day were transferred to the long-day conditions (treatment 12→18), their mortality was significantly ($\chi^2 = 9.9$, d.f. = 1, $P = 0.002$) higher than that in females that were permanently reared at short day (treatment 12→12) (Fig. 1).

The effect of diet on survival was highly significant ($P < 0.001$) under all combinations of photoperiods. In particular, mortality in females fed on S + 5A diet was much higher than that in females fed on other diets. Feeding on sugar diet also reduced survival but only in females kept at the long-day conditions. Moreover, the mortality of individuals transferred from short to long day (regimen 12→18) and fed on S diet was not significantly lower than in those fed on S + 5A diet (Fig. 1).

The proportion of ovipositing females

The proportion of females that started to lay eggs during the 60 days of the experiment was significantly dependent both on the photoperiodic regimen and on diet (Fig. 2). Analysis of the total data set (with the exception of females fed on S diet, none of which oviposited) by the chi-square test with the Mantel–Haenszel adjustment showed that, as expected, when the adult females were kept under long-day photoperiod, they started to lay more often than those kept under short day ($\chi^2 = 18.7$, $P < 0.001$ and $\chi^2 = 32.3$, $P < 0.001$ with the larval photoperiod and with diet used as strata variable, correspondingly). Separate treatment of the data for individuals that have

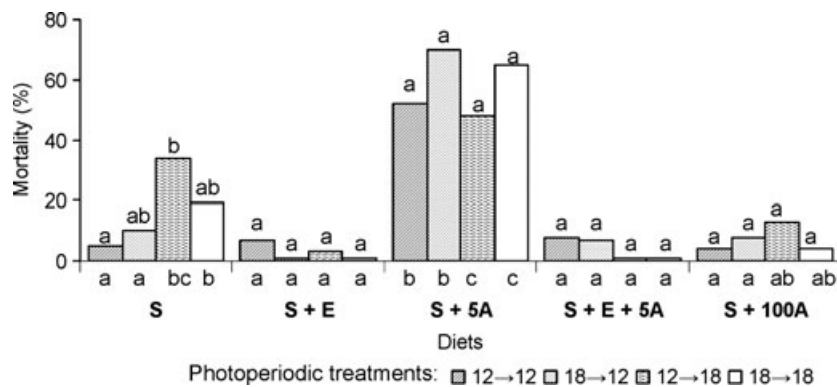


Fig. 1 Mortality of *Harmonia axyridis* females in relation to diet and photoperiodic treatment. Bars indicate the proportion of females that did not survive till the end of the experiment (over 60 days after emergence). Diets are indicated along the x-axis: 'S' – 50% sugar solution; 'S + E' – sugar solution and eggs of *Sitotroga cerealella*; 'S + 5A' – sugar solution and 5 aphids provided daily; 'S + E + 5A' – sugar solution and *S. cerealella* eggs and 5 aphids provided daily; 'S + 100A' – sugar solution and ca 100 aphids provided daily. Photoperiodic treatments: '12→12' – both larvae and adults were kept under short day (L : D = 12 : 12); '18→18' – both larvae and adults were kept under long day (L : D = 18 : 6); '12→18' – larvae were kept under short day and adults were kept under long day; '18→12' – larvae were kept under long day and adults were kept under short day. See Materials and Methods for more details. Different letters above bars indicate significant ($P < 0.05$, chi-square test) difference between females fed on the same diet under different photoperiodic treatments. Different letters below bars indicate significant ($P < 0.05$, chi-square test) difference between females fed on different diets in the same photoperiodic treatment.

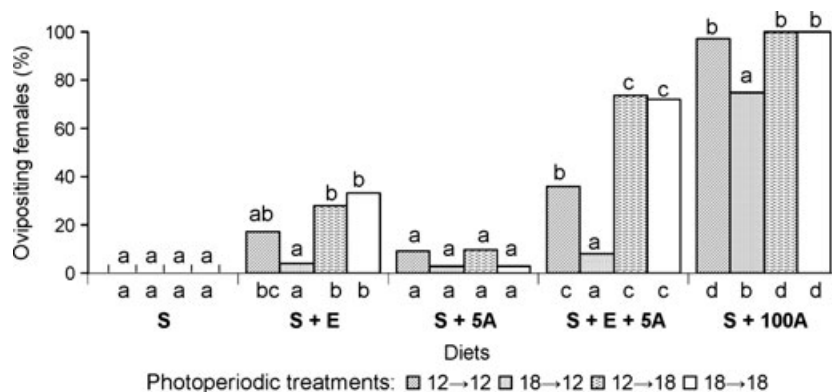


Fig. 2 The proportion of ovipositing females of *Harmonia axyridis* in relation to diet and photoperiodic treatment. Bars indicate the proportion of females that started to lay eggs before the end of the experiment (over 60 days after emergence). Other explanations as in Fig. 1.

developed at short- and long-day conditions (diet was a strata variable) showed that when pre-imaginal development occurred under long day, the effect of the adult photoperiod was much more significant than in females that have developed under short day ($\chi^2 = 30.7$, $P < 0.001$ and $\chi^2 = 5.9$, $P = 0.015$, for females that have developed under long and under short day, correspondingly).

The effect of the larval photoperiod was also significant: $\chi^2 = 4.0$, $P = 0.047$ and $\chi^2 = 6.0$, $P = 0.014$ with the adult photoperiod and with diet used as strata variable, correspondingly. The pattern of this larval photoperiodic response was opposite to that of the adult photoperiodic reaction: pre-imaginal development under long day caused a decrease in the proportion of ovipositing females. Further analysis of the data showed that this effect was very strong when adult females were kept under short day (treatments 12→12 and 18→12, $\chi^2 = 12.2$, $P < 0.001$, diet was used as a strata variable), while in females kept under long day (treatments 12→18 and 18→18), it was practically absent ($P = 0.99$), this is also clearly seen in Fig. 2.

Comparison of photoperiodic responses of females fed on diets S + E and S + E + 5A revealed the same general pattern: the minimum proportion of ovipositing females was recorded when larvae developed under the long-day conditions, but adults were transferred to the short-day photoperiod (treatment 18→12); the individuals kept permanently at the short day (treatment 12→12) significantly more often started to lay eggs, while the long-day adult photoperiod (treatments 12→18 and 18→18) resulted in the maximal proportion of egg-laying females. In lady beetles fed on diet S + 100A, the minimum proportion of ovipositing females was also recorded in the treatment 18→12, but the difference between the other three photoperiodic regimens was not significant (the proportion of ovipositing females closely approached 100%). When females fed on diet S + 5A, the percentage of ovipositing individuals was

very low and photoperiodic effects were not significant, while females fed only with sugar solution (diet S) never laid an egg.

The effect of diet on the proportion of ovipositing females was very strong but still dependent on the photoperiodic regimen. As seen in Fig. 2, under all combinations of photoperiodic conditions of larval development and adult maturation, but excluding the treatment 18→12, the percentage of females that started to lay eggs before the end of the experiment gradually increased in the following succession of diets: S < S + 5A < S + E < S + E + 5A < S + 100A. Although separate pairwise comparisons of the data for different photoperiodic treatments did not reveal significant differences between females fed on diets S and S + 5A, the difference between the pooled data for the two above diets was highly significant: $\chi^2 = 7.0$, $P = 0.008$. For the other diets, pairwise differences were almost always significant (Fig. 2). In the treatment 18→12, however, the results were somewhat different: the proportions of egg-laying individuals among females fed on diets S + 5A, S + E and S + E + 5A were practically the same, while S + 100A diet caused a sharp increase in the proportion of ovipositing females.

Maturation time of ovipositing females

TWO-factor ANOVA of the ranked data showed that the duration of maturation period in females that started to lay eggs before the end of the experiment ($n = 185$) was significantly ($P < 0.001$) dependent both on diet and on photoperiodic treatment although the photoperiodic effect ($F = 6.7$) was much smaller than that of the diet ($F = 25.4$). The interaction of these factors was relatively weak and only marginally significant ($F = 2.0$, $P = 0.044$).

Separate ANOVA of the data for different diets showed that the photoperiodic effects on the maturation time of females fed on diets S + E, S + 5A and

S + E + 5A were not statistically significant, possibly, because of the small number of females that started to oviposit on these diets ($n = 24$, $n = 8$, and $n = 50$, correspondingly). Treatment of the pooled data for the above three diets showed that females that have developed and were kept under long day (treatment 18→18) started to lay eggs significantly ($P = 0.013$, the Tukey HSD test) faster than those developed and kept under short day (treatment 12→12). As seen in Fig. 3, this trend is also present in females fed on diet S + 100A: the duration of the maturation period in the treatment 12→12 was significantly ($P < 0.003$, the Tukey HSD test) higher than in the other treatments.

The one-way ANOVA of the ranked pooled data of all photoperiodic treatments showed that the effect of diet on the maturation time was rather clear and, as may be expected, was quite opposite to that on the proportion of egg-laying females (comp. Figs 2 and 3). The longest average period of maturation was recorded in females fed on diets S + E and S + 5A; the Tukey HSD test showed that the difference between the effects of these two diets was not significant. In females fed on the mixture of the above diets (S + E + 5A), the maturation period was significantly ($P < 0.02$) shorter, while the difference between the 'best' diet S + 100A and other diets was very highly significant ($P < 0.001$). Separate pairwise comparisons of the 'diet * photoperiodic treatment' combinations (Fig. 3) showed that only diet S + 100A significantly differed from other diets. Moreover, for the treatment 18→12, even this difference was not significant, possibly because of the very small number of ovipositing females.

The proportion of maturing females

Oviposition, however, is only a final stage of a long process of oogenesis, while dissection can reveal the very beginning. As seen in Fig. 4, the photoperiodic effect on the proportion of maturing females (i.e. those that did not lay eggs during the experiment but showed various stages of oogenesis at dissection) was rather clear when beetles fed on diets S + E and S + E + 5A. In both cases, the highest percentage of maturing individuals was recorded among females kept under the long-day conditions (treatments 12→18 and 18→18). When adults were kept under short-day photoperiod, long-day conditions during their pre-imaginal development caused a further decrease in the proportion of maturing females (comp. treatments 18→12 and 12→12) although this effect was significant only with diet S + E. As for other diets, none of the females fed on diet S have even started to mature; in females fed on diet S + 5A, the photoperiodic effect was not statistically significant, while all females fed on diet S + 100A (excluding the treatment 18→12) have either died or started to lay eggs before the end of the experiment.

The effect of food on the proportion of maturing but not yet ovipositing females (photoperiodic treatment being the same) was clear only in beetles fed on diet S (none have started maturing). In addition, diets S + E and S + E + 5A seem to induce maturation more often than diet S + 5A, although this effect was significant only under long-day photoperiods (Fig. 4). As was noted above, some females have died before the end of the experiment (Fig. 1) and therefore have not been dissected. However, the effect of diet can be

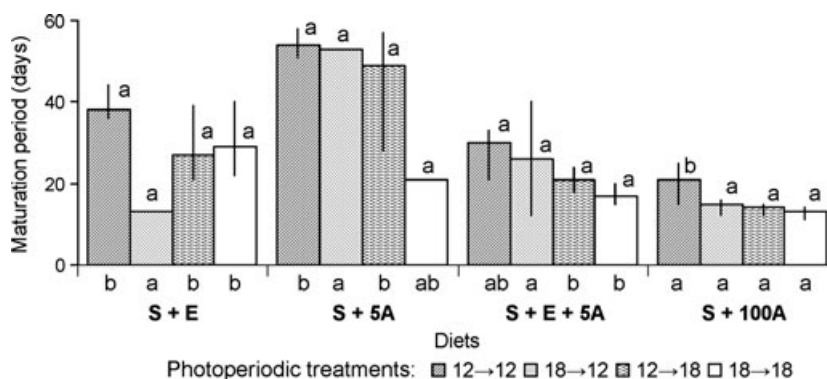


Fig. 3 The duration of the maturation (pre-oviposition) period of ovipositing females of *Harmonia axyridis* in relation to diet and photoperiodic treatment. Bars indicate the duration of the maturation period (medians and quartiles) for females that started to lay eggs before the end of the experiment (over 60 days after emergence). Data on females fed on sugar solution (diet S) are not indicated as none of these females have started oviposition. Different letters above bars indicate significant ($P < 0.05$, the Tukey HSD test of the ranked data) difference between females fed on the same diet under different photoperiodic treatments. Different letters below bars indicate significant ($P < 0.05$, the Tukey HSD test of the ranked data) difference between females fed on different diets in the same photoperiodic treatment. Other explanations as in Fig. 1.

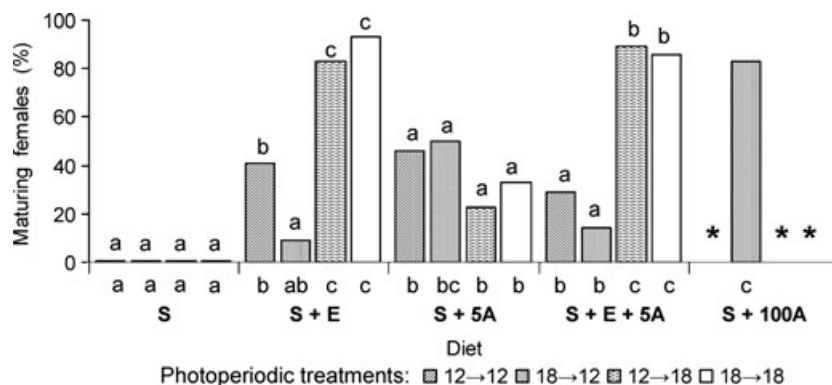


Fig. 4 The proportion of maturing females of *Harmonia axyridis* (as determined by dissection in 60 days after emergence) in relation to diet and photoperiodic treatment. Bars indicate the proportion of females that did not lay eggs during the experiment but showed various stages of oogenesis at dissection. Asterisk means no data (all females laid eggs before the end of the experiment). See Materials and Methods for more details. Other explanations as in Fig. 1.

detected in these females as well. In particular, none of females fed on diets S, S + E and S + 5A have laid eggs before the death, while 25% and 63% of females fed on diets S + E + 5A and S + 100A, correspondingly, have oviposited ($P < 0.001$, chi-square test).

Fecundity of ovipositing females

Two-factor ANOVA showed that the mean daily number of eggs laid by ovipositing female (total number of eggs laid divided by the number of days from the laying of the first egg to the end of the experiment or to the death of the female) was strongly ($n = 185$, $F = 28.5$, $P < 0.001$) dependent on diet while the effect of photoperiodic treatment and the interaction of these factors were not significant ($P > 0.7$). As seen in Fig. 5 where the pooled data of all photoperiodic treatments are shown, the mean daily number of eggs laid per ovipositing female gradually increased in the same succession of diets which was recorded for the percentage of egg-laying

females: $S + 5A < S + E < S + E + 5A < S + 100A$, although the difference between the first two diets was not significant.

Discussion

Photoperiodic responses

As was noted in the Introduction, photoperiodic reactions of *H. axyridis* have been studied by different authors. Particularly, it has been demonstrated that short days caused a decrease in the proportion of ovipositing females and/or an increase in the duration of the pre-oviposition period (Voronin 1965; Sakurai et al. 1988; Ongagna and Iperti 1994; Hodek and Honěk 1996; Iperti and Bertrand 2001; Berkvens et al. 2008b; Reznik and Vaghina 2011). Our data (Figs 2 and 3) agree well with these studies. Moreover, from Fig. 4, it is clear that the proportion of maturing (although not yet ovipositing) females also increased with day length, which has not been described earlier.

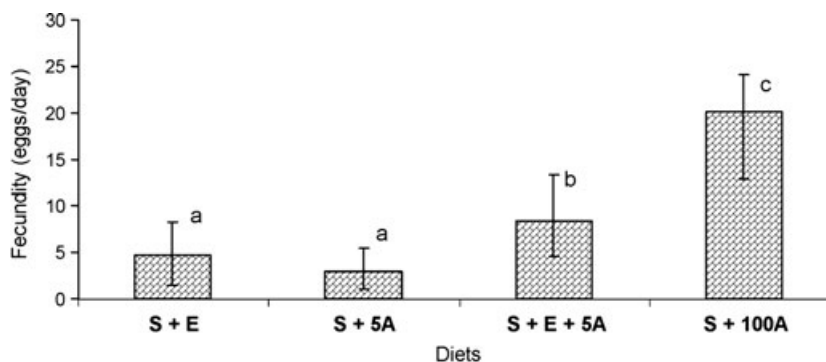


Fig. 5 The average daily fecundity of ovipositing females of *Harmonia axyridis* in relation to diet and photoperiodic treatment. Bars indicate the number of eggs laid per female per day (medians and quartiles) for females that started to lay eggs before the end of the experiment (over 60 days after emergence). Data on females fed on sugar solution (diet S) are not indicated as none of these females have started oviposition. Pooled data for all photoperiodic treatments are given, different letters above bars indicate significant ($P < 0.05$, the Tukey HSD test of the ranked data) difference between females fed on different diets. Other explanations as in Fig. 1.

Another novelty of the present study is a clear-cut reaction to the direction of changes in the day length or a 'stepwise photoperiodic response': when larvae and pupae developed at long day (L : D = 18 : 6) and adults were transferred to short day (L : D = 12 : 12), the decrease in the proportion of ovipositing (Fig. 2) and maturing (Fig. 4) females was even stronger than that in individuals that were permanently kept under short-day conditions. This type of photoperiodic response has been observed in various insect species (Tauber et al. 1986; Danks 1987; Zaslavski 1988; Saunders 2002) including coccinellids (Simakova 1981; Hodek and Honěk 1996 and many references therein). However, it has not been earlier demonstrated for *H. axyridis*. Moreover, in most of the above-cited studies, adult lady beetles were sequentially kept at different photoperiods, while in our experiment, long and short days were applied to pre-imaginal and then to adult stages. This larval photosensitivity has been demonstrated for relatively few coccinellids (Zaslavski 1970; Tadmor and Applebaum 1971; Storch and Vaundell 1972; Hodek and Honěk 1996).

It should be also noted that the effect of the direction of changes in the day length can be revealed in the induction of maturation but not in the duration of the maturation period. Females in the treatments 18→12 started to lay eggs much more rarely than those in the treatments 12→12, but their average maturation periods were shorter (comp. Figs 2 and 3).

Trophic effects

The effects of diet on the proportion of ovipositing females (Fig. 2), on the average maturation period (Fig. 3) and on the mean fecundity (Fig. 5) have been studied in many species of Coccinellidae. As in the present study, more 'suitable' or 'rich' diet usually ensures faster maturation, higher fecundity and higher proportion of egg-laying females (Wipperfürth et al. 1987; Hodek and Honěk 1996; Zaslavski et al. 1998; Dixon 2000; Evans 2000; Soares et al. 2004, 2005; Evans and Gunther 2005; Osawa 2005; Berkvens et al. 2008a,b; Sighinolfi et al. 2008; Omkar et al. 2010; Agarwala and Bhowmik 2011). The mean pre-oviposition periods and the mean daily fecundity of *H. axyridis* reported by different authors (Abdel-Salam and Abdel-Baky 2001; Lanzoni et al. 2004; Mignault et al. 2006; Reznik and Vaghina 2011; Santos et al. 2009; Castro et al. 2011; Stathas et al. 2011) were rather similar to those observed in the present study.

As seen in Fig. 1, an adding of five aphids per day to the plain sugar diet caused a sharp increase in adult mortality. Possibly, consumption of even such a low

number of aphids can serve as an environmental cue regulating the transition from diapause to the reproductive state, but on the other hand, the nutritional value of this additive is too low to support long-term survival of active (non-diapausing) females. This non-linear effect of the mixed diet on the adult survival is a manifestation of a more general rule: the range of factors that can induce an active reproductive state (cue effect) is much wider than the range of factors that can support the whole process of oogenesis (direct effect). This conclusion is also suggested by the comparison of Figs 2–4: feeding on 'poor' diets S + E and S + E + 5A resulted in a slow maturation and in a low percentage of egg-laying females, but dissection showed that most of non-laying females (particularly those kept under long-day conditions) have started maturation. We conclude that only the plain sugar diet could not serve as an environmental cue to trigger reproductive activity, and this cannot be explained by that sugar is nutritionally inadequate for egg production, as the first stages of reproductive maturation do not require protein feeding and can be triggered even by smell of aphids (Zaslavski et al. 1998).

Relatively low proportion of maturing females fed on diet S + 5A can be most probably explained by non-random mortality: with this diet, non-diapausing individuals have much less chances to survive until dissection. Similar stimulating effects of scarce prey have been recorded for some other predatory coccinellids: dissections showed that reactivation from the trophic diapause was induced irrespective of the quantity of prey while the amount of food affects the rate of maturation and number of eggs laid (Hodek and Honěk 1996; Zaslavski et al. 1998; Vaghina 2004; Omkar et al. 2010; Agarwala and Bhowmik 2011).

These non-linear effects, however, can be found only in stimulating cue action of food. When nutritional action (e.g. trophic effect on the mean daily fecundity) was studied, combination of diets resulted in simple linear summation of their results: the average daily fecundity of females fed on S + E + 5A diet was practically equal to the sum of those of females fed on diets S + E and S + 5A (Fig. 5). Similar effects of mixed diets have been recorded for different species of predatory coccinellids (Hodek and Honěk 1996; Evans 2000; Soares et al. 2004, 2005; Evans and Gunther 2005; Berkvens et al. 2008a,b).

Summarizing the results of the present study and the literature analysis, we conclude that our experiments allowed to clearly distinguish between direct (nutritional) and cue (informational) effects of diet on reproduction of *H. axyridis*. As was hypothesized, cue effect (reproductive activation) was achieved almost

independently of the number of prey consumed, while feeding on sugar did not trigger reproductive activity. Nutritional effects (changes in the rate of maturation and fecundity), on the contrary, were sensitive both to the quality and quantity of food, mixed diet seemed to produce cumulative effect (summation). In natural conditions, in mass rearing, and in simple laboratory experiments, mostly nutritional effects are observed as variations in the egg-laying intensity. Cue effects, however, can be also detected by changes in survival and, possibly, in the readiness to start oviposition with the advent of favourable feeding conditions.

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