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Can the intensity of diapause be expressed in terms of metabolic rate?

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Abstract. We estimated the effect of photoperiod at a temperature of 25±1 °C on diapause incidence, developmental rate and pre-oviposition period of the linden bug, *Pyrrhocoris apterus* (Linnaeus, 1758) (Heteroptera: Pyrrhocoridae). At 12L (light):12D (darkness), 14L:10D, 15L:9D 100% of the females entered diapause, at 16L:8D it was only slightly lower, at 16.5L:7.5D (critical photoperiod) it was 54%, at 17L:7D it was near to 0% and at 18L:6D it was 0%. Larval development was longest at conditions near to the critical photoperiod. At photoperiods of 16L:8D and 16.5L:7.5D some of the females that entered diapause had a longer larval development than some of the non-diapause females. The duration of the pre-oviposition period of diapause females activated keeping them at a photoperiod of 18L:6D depended on the photoperiod that previously induced diapause. The longest pre-oviposition period was recorded for females kept at 12L:12D and the shortest at 15L:9D, 16L:8D or the critical photoperiod of 16.5L:7.5D. Conversely, oxygen consumption of adults kept at photoperiods of 12L:12D, 15L:9D and 16L:8D was similar. Only females kept at the critical photoperiod 16.5L:7.5D showed a slightly enhanced oxygen consumption. If the oxygen consumption is measured continuously during the photoperiodic activation of females from 12L:12D, it remained low and stable until a short period before oviposition when a higher metabolic rate associated with the maturation of oocytes was recorded. If the duration of pre-oviposition period of activated females is accepted as a criterion of diapause intensity, it may be concluded that oxygen consumption and diapause intensity are mutually independent parameters.

Key words. Diapause, diapause development, diapause incidence, diapause intensity, oxygen consumption, photoperiod, temperature, *Pyrrhocoris apterus*.

INTRODUCTION

Diapause is the most important of the seasonal adaptations that synchronize the development of insect populations with suitable conditions (presence of food, suitable climatic conditions).

From a long list of definitions of diapause we have chosen that formulated by Danks (1987: 9–10): In contrast to quiescence, diapause is a more profound suppression of development "that routes the metabolic programme of the organism away from direct developmental pathways" into those "not controlled simply by the direct action of environmental factors, and which in nature precedes the advent of adverse conditions." Thus the adaptive functions of diapause are: (1) to synchronize the development of active/feeding stages with favourable conditions, and (2) to enhance the survival potential during unfavourable periods (Hodek 2012: 276).

While there are many general studies on diapause there are problems with the interpretation of the results, mostly due to inconsistencies between the predictions of general theory and empirical data. Intensity of diapause (and the methods for measuring it) is one such important but controversial feature. More insight into the phenomenon and term "diapause intensity" is needed, which can be achieved by comparing experimentally the effects of factors that do not differ greatly, but may have different qualitative effects. Thus, e.g., insects may be reared at day lengths differing

little in absolute values but which produce a marked difference due to their relative position to a particular threshold.

Effect of photoperiod on larval developmental rate

Photoperiod affects the rate of development in many insect species. Diapause inducing photoperiods usually decrease the developmental rate before the onset of diapause (Danks 1987, Saunders 2002). An unusual phenomenon is reported in *Pyrrhocoris apterus*: The mean duration of larval development increases at photoperiods around the critical value for diapause regulation (Saunders 1983, Numata et al. 1993). The significance of this is not clear. Similarly Lopatina et al. (2007) show that the larval development of *P. apterus* at relatively high temperatures (above 24–25 °C) proceeds faster under long than under short days, which should be advantageous at the height of summer. At relatively low temperatures (below 24–25 °C) larvae develop faster under short than under long days. This is likely to be advantageous at the end of summer as it would enable larvae to reach the adult stage, which can successfully overwinter. The interaction between temperature and photoperiod in the regulation of the rate of development occurs also in the pentatomid *Nezara viridula*: at a high temperature (25 °C) development is faster under an intermediate photoperiod then under short or long days, while the opposite is recorded at a low temperature (20 °C) (Musolin & Numata 2003). According to Honek (1983) artificially slowed down development in the last larval instar of *P. apterus* produced diapause even under long days.

One aim of the present study was to ascertain the relation between diapause induced at different photoperiods and the duration of larval development of individual insects at 25 °C. At intermediate photoperiods there is a tendency for individuals that do not enter diapause to develop faster.

Diapause intensity and metabolic rate

Although the diapause response has a qualitative character ("all or none" response), diapause of different intensities can be induced by modifying the inducing factors, particularly photoperiod. Perhaps the first such finding was recorded by Tauber & Tauber (1972) in *Chrysopa carnea* (now *Chrysoperla plorabunda*). In some cases e.g. in *Manduca sexta* (Bell et al. 1975), *Aphidoletes aphidimyza* (Havelka 1980) and *Nineta flava* (Canard 1983) diapause is more intense when induced by photoperiods near the critical value for inducing diapause. In other species, e.g. in *Ostrinia nubilalis* (Beck 1980), *Riptortus clavatus* (Numata & Hidaka 1983, Nakamura & Numata 2000) and *Drosophila auraria* (Kimura 1990), the most efficient photoperiods (shortest photophases in long-day insects and longest photophases in short-day insects) induce the most intense diapause. Other environmental factors, such as temperature, population density etc., may also modify diapause intensity (Tauber et al. 1986, Danks 1987, Tauber & Tauber 2015).

Diapause intensity is often described in quite vague terms, as weak or deep/strong diapause, but even when the word intensity is used it is not clear what this term means. The intensity has mostly been measured "a posteriori" as the length of time required for the resumption of development (Hodek 1971a, b, Danks 1987, Kostal et al. 2008).

However, we are usually interested in the actual state of a diapausing insect not a "a posteriori" measure of its condition. Attempts have been made to measure the intensity of diapause using metabolic rate (Hodek & Hodkova 1981). This is logical because a lowered metabolic rate is considered to be the essential symptom of diapause as an adaptive phenomenon (Tauber et al. 1986, Danks 1987). In two lepidopterans, *Lacanobia oleracea* and *Mamestra brassicae*, oxygen consumption has been used as an indicator of diapause intensity induced by different photoperiods at 25 °C (Varjas & Saringer 1998).

Interspecific comparisons indicate that diapause intensity is inversely related to oxygen consumption (Beck 1980). However, this relationship is less clear if diapause intensity is measured

in this way for diapausing individuals of a species that have experienced different "histories" of inducing factors, etc. Diapause intensity may vary at its onset due to differences in the inducing factors and also changes (usually diminishes) with the progress of diapause development (Hodek 1983, 2002).

Oxygen consumption at 22 °C of the solitary bee, *Osmia lignaria,* overwintering at 0, 4 and 7 °C is similar over the first ~100 days, but than starts to diverge with an exponential increase occurring earliest in those kept at 7 °C (Sgolastra et al. 2010). The authors conclude that diapause was weakest in bees that overwintered at 7 °C. Alternatively, 7 ºC may be the optimum temperature for diapause development and the increase in metabolism at 7 °C was probably because these bees were in post-diapause and already developing. Respiration recorded at 25 °C for *P. apterus* and *Aelia acuminata* that were previously diapausing at 15 °C increases slightly with time, but there is no significant correlation between the preoviposition period of individual females and their oxygen consumption, which indicates there is no clear relationship between diapause intensity and oxygen consumption (Hodek & Hodkova 1981). In *P. apterus* the oxygen consumption is even greater at a thermoperiod of 25 °C day 15 °C night than at a constant 25 °C, although the diapause intensity measured in terms of the duration of the pre-oviposition period is greater in the former case (Kalushkov et al. 2001).

Denlinger (2002) states that the intensity of diapause is determined by qualitative differences in gene expression, which do not affect the metabolic rate. Changes in level of transcription of genes coding for AR and SoDH closely match changes in diapause intensity in *P. apterus* (Kostal et al. 2008).

Several of the examples above indicate that the notion of the diapause intensity expressed in terms of the duration of diapause development is not accepted uniformly. Therefore the other aim of this study was to determine the relationship between the intensity of adult diapause and oxygen consumption in *P. apterus.* We did two experiments: (1) Females induced to diapause by keeping them at several different photoperiods (a) had their oxygen consumption recorded once before transfer to an activation photoperiod (b) after which the duration of diapause was measured. (2) Females induced to diapause at one photoperiod had their oxygen consumption measured repeatedly until the onset of reproduction.

MATERIAL AND METHODS

Insects

We used *Pyrrhocoris apterus* bugs from a culture kept in the Entomological Institute, Czech Academy of Sciences, which originated from bugs collected in the village of Chelcice near Ceske Budejovice (49° N) and reared at 25±1 °C in incubators at a regulated photoperiod. At this temperature and a 18L:6D photoperiod the pre-adult development (egg and five larval instars) lasted 4–5 weeks. The bugs were reared on linden seeds and water in 0.5 liter jars covered with nylon tissue.

Duration of development was measured as the number of days from oviposition to adult emergence.

The eggs laid during the preceding 24 hr were transferred to six incubators kept at 25±1 °C and with one of the following range of photoperiods: 12L:12D, 14L:10D, 15L:9D (short days, SD), 16L:8D, 16.5L:7.5D (intermediate photoperiods) and 18L:6D (long day, LD). Three groups, each of 100 eggs were kept at each photoperiod. The newly emerged adults were isolated daily and the duration of their larval development recorded. Experimental females were reared individually in Petri dishes and the number of egg they laid monitored. Females lay infertile eggs in the absence of males. Females were considered to be non-diapausing if they started ovipositing within two weeks of emerging as an adult. Diapausing females did not lay any eggs for at least 1.5–2 months or before the end of the experiment.

Diapause intensity was evaluated by measuring the duration of pre-oviposition period, after transfering diapausing females to 18L:6D at the age of 1.5–2.0 months, i.e. when diapause intensity was greatest (Hodek 1978). One day before the transfer to 18L:6D, females were weighed using an analytical balance (Sartorius) and their oxygen consumption measured.

Oxygen consumption

The metabolic rate was measured manometrically as oxygen consumption using a Warburg respirometer (Slama 1964). Respiratory vessels of about 10 ml volume containing 5% potassium hydroxide to absorb carbon dioxide and a small piece of wet cotton to maintain a constant water vapour pressure were used. The oxygen consumption recorded for each individual at rest at a temperature of 26 °C is the average of three 0.5 h readings.

Statistics

Differences between parameters were analyzed using a one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests (development time, weight, pre-oviposition, oxygen consumption). Linear regression (weight, oxygen consumption) was computed using GraphPad Prism 5 software. Statistics are given in the legends of the Figures.

RESULTS

Effect of photoperiod on diapause incidence, development rate and adult weight

At 12L:12D, 14L:10D, 15L:9D all females entered diapause and at 16L:8D diapause incidence was only slightly lower. At 18L:6D diapause incidence was 0% and at 17L:7D it was nearly 0%. At 16.5L:7.5D (critical photoperiod) 54% of females entered diapause. The incidence of diapause can be modified by temperature at the critical photoperiod of 16.5L:7.5D as an increase in temperature of 2–3 °C greatly lowered the percentage of diapause females from 54% to 7% (Figs. 1, 2).

The pre-adult development was longer at photoperiods near the value critical for diapause induction (Fig. 1). It was longest at 16L:8D, when it lasted 44.1 days and thus 10 days longer than in the typical short day of 12L:12D (34.7 days) or in the diapause preventing 18L:6D (34.3 days).

The comparison of diapausing and reproducing females from photoperiodic conditions around the critical photoperiod revealed a tendency for those females that entered diapause to take longer to complete their development: at 16.5L:7.5D the development of females that entered diapause lasted 43.2 days, while that of the non-diapausing females was 37.8 days (Fig. 2). The tendency of non-diapausing females at 16L:8D and 16.5L:7.5D to develop more quickly is also seen if the relation between length of development and diapause is expressed for individual females (Fig. 2).

Adult diapause in *P. apterus* is deepest 1.5–2 months after induction (Fig. 4 in Hodek 1983). The weight of diapause females of this age from 16L:8D (74.4 mg, n=53) was significantly greater ($P<0.005$) than that of diapause females from 12L:12D (68.3 mg, n=71). However, the relation

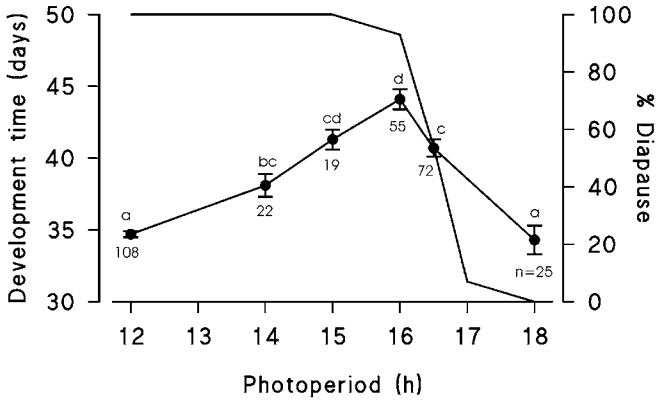

Fig. 1. Effect of photoperiod on development time. Values are means±SEM. Significantly different values (One-way ANOVA, Tukey's Multiple Comparison Test) are marked by different letters. Full line indicates diapause incidence.

between the weight and duration of larval development of individuals within the groups kept under different photoperiods showed an opposite tendency, i.e. lowering of weight with prolongation of development (Fig. 3).

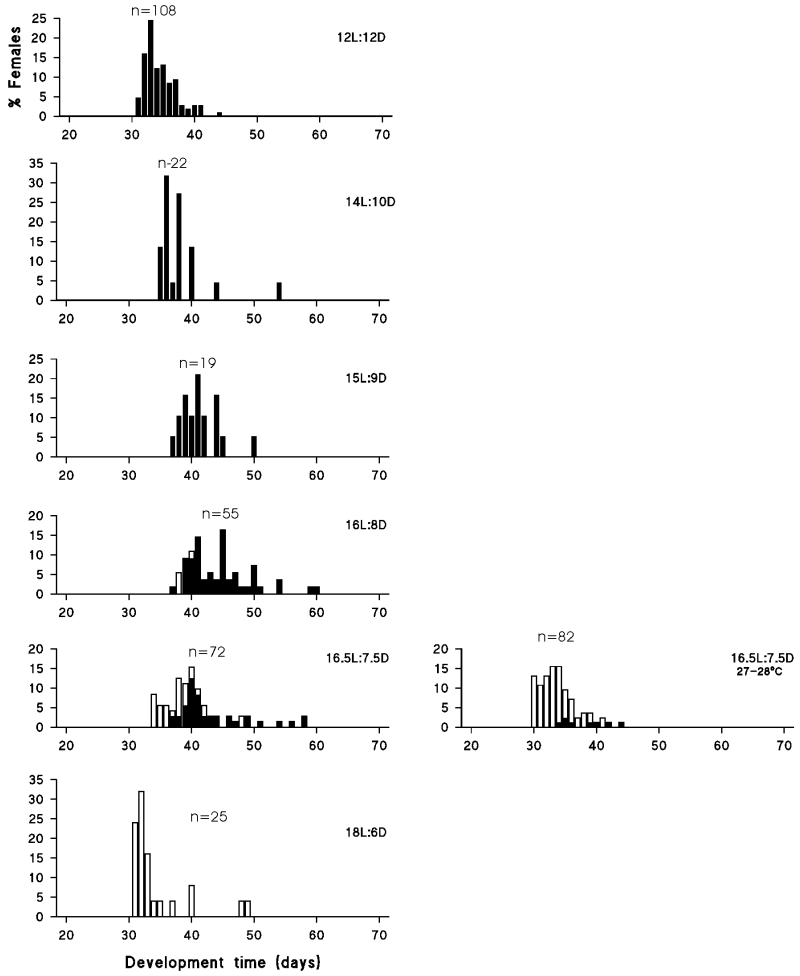

Fig. 2. Relationship between development time and diapause for individual females. Left – 25±1 °C, right – 27–28 °C, solid columns – diapause females, open columns – non-diapause females. For mean values see Fig. 1.

Effect of diapause promoting photoperiod on reproduction after diapause

The duration of the pre-oviposition period of adults aged 1.5–2 months, recorded after transfer to long days, was longest for females reared as larvae and young females at 12L:12D (32.6 days), slightly shorter at 14L:10D (29.4 days) and shortest for females reared at photoperiods at or near

the critical day-length (22.5 days at 15L:9D, 21.6 days at 16L:8D, 22.2 days at 16.5L:7.5D) (Fig. 4).

If the duration of the pre-oviposition period is accepted as a criterion of diapause intensity, then the least intense diapause was induced at intermediate photoperiods.

Effect of three diapause promoting photoperiods on oxygen consumption during (deep) diapause

The mean consumption of oxygen by females that had been in diapause for 1.5–2 months, i.e. before their transfer to a different photoperiod, was very similar in three experimental photoperiods: 0.326 ml/g/h at 12L:12D, 0.348 ml/g/h at 15L:9D and 0.331 ml/g/h at 16L:8D (Fig. 5). The intensity of diapause, when measured as duration of pre-oviposition period, was, however, about 10 days shorter at 16L:8D than at 12L:12D or 14L:10D (chapter 3.2., Fig. 4). At the critical day length of 16.5L: 7.5D, oxygen consumption was significantly higher (0.471 ml/g/h) than at the former photoperiods, although diapause intensity measured as pre-oviposition period was similar to that at photoperiods 15L:9D and 16L:8D (Fig. 4). The absence of a correlation between the length of pre-oviposition period and oxygen consumption of individual females (Fig. 6) indicates that these two parameters are probably independent.

Metabolic rate of diapausing females exposed to photoperiodic activation

There is a decrease in diapause intensity during the course of diapause development when exposed to long days that induce reproduction, i.e. photoperiodic activation of diapause. Oxygen consumption of 16 diapausing females aged between 12–16 days was measured for 10 days at 12L:12D. After a further 5 days these females were transferred to 18L:6D and their oxygen consumption measured up until they started laying eggs. Although the intensity of diapause decreased, oxygen consumption was low and stable until about 7–8 days before oviposition when it started to increase

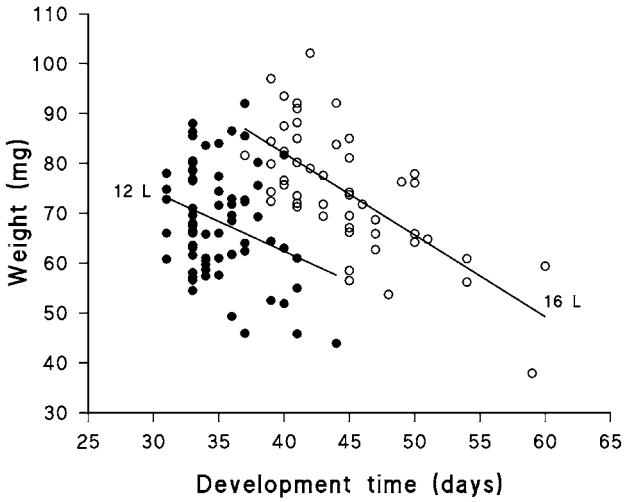

Fig. 3. Relationship between weight and development time. Solid circles – 12L:12D, linear regression: r=–0.38, P<0.05. Open circles – 16L:8D, linear regression: r=–0.68, P<0.0001. Only diapause females are included.

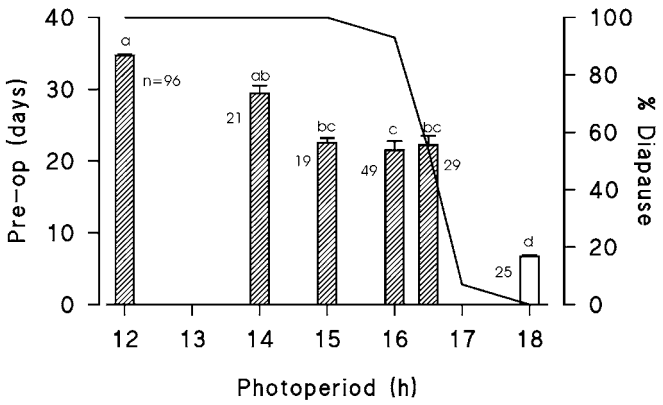

Fig. 4. Effect of inducing photoperiod on pre-oviposition period. Values are means±SEM. Significantly different values (One-way ANOVA, Tukey's Multiple Comparison Test) are marked by different letters. Diapause females from photoperiods between 12L:12D and 16.5:7.5D are indicated by hatched columns. Non-diapause females from 18L:6D are indicated by the open column. Full line indicates diapause incidence.

sharply. A period of 7–8 days at long days and 25 °C is required for maturation of oocytes. Similar trends were recorded for all 16 females, with the pre-oviposition period at 18L:6D ranging from 15 to 28 days. The results for the three females with the shortest, intermediate and longest pre--oviposition periods, respectively, are depicted in Fig. 7. In all cases, the increase in metabolic rate is associated with the maturation of oocytes and not with the decrease in diapause intensity.

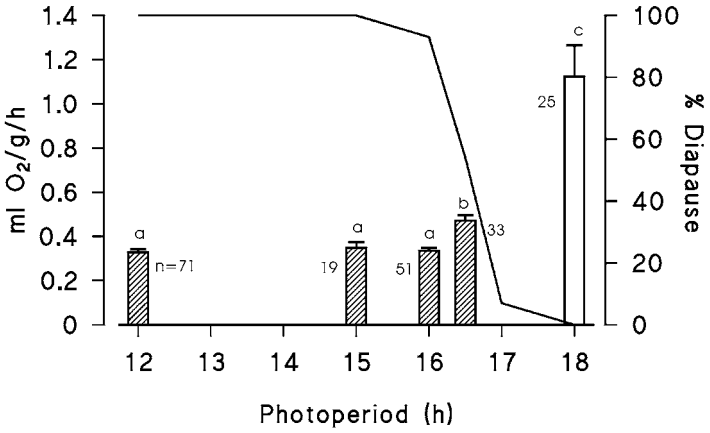

Fig. 5. Effect of inducing photoperiod on the oxygen consumption of adults. Values are means±SEM. Significantly different values (One-way ANOVA, Tukey's Multiple Comparison Test) are marked by different letters. Diapause females from 12L:12D to 16.5:7.5D are indicated by hatched columns. Females from 18L:6D are indicated by the open column and are all non-diapausing individuals. Full line indicates diapause incidence.

DISCUSSION

Photoperiod critical for diapause induction

The critical photoperiod recorded for the population of *P. apterus* from southern Bohemia (49° N) (54% diapause at 16.5L:7.5D) is similar to that reported for a population from northern Bohemia (Hodek 1968). Saunders (1983) reports a value 15.75L for Czech insects from a culture at the Institute of Entomology CAS, which were, however, reared for several generations at 16L:8D. A Russian population from Belgorod, which is at a similar latitude (50° N), has an identical critical photoperiod, close to 16.5L:7.5D at 25 °C (Goryshin & Volkovich 1984). A very similar critical photoperiod of 17L:7D at 24.5 °C is reported by Numata et al. (1993) for a Russian population (50° N).

The well-known effect of an increase in temperature shortening the critical day-length (Saunders 2002) is corroborated by the results of the present study in which the incidence of diapause at 16.5L:7.5D decreased from 54% to 7% when the temperature was increased by 2–3 °C (Fig. 2). Similarly, the critical photoperiod of the Belgorod population decreases with an increase in temperature from 17L:7D to 15.5L:8.5D when temperature is increased by 2.5 °C (Numata et al. 1993).

Relation between the larval developmental rate and diapause

We confirmed the earlier findings (Saunders 1983, Numata et al. 1993) of a long larval development at intermediate photoperiods (at or near the critical level) and a short larval development at both short and long day-lengths. This can be accounted for in two ways.

(*1*) *Homogenization of life cycles*

Larvae that emerge from the eggs in early spring are exposed to several weeks of photoperiods near the critical level (14.5L:9.5D – 16.5L:7.5D) and consequently develop slowly, whereas

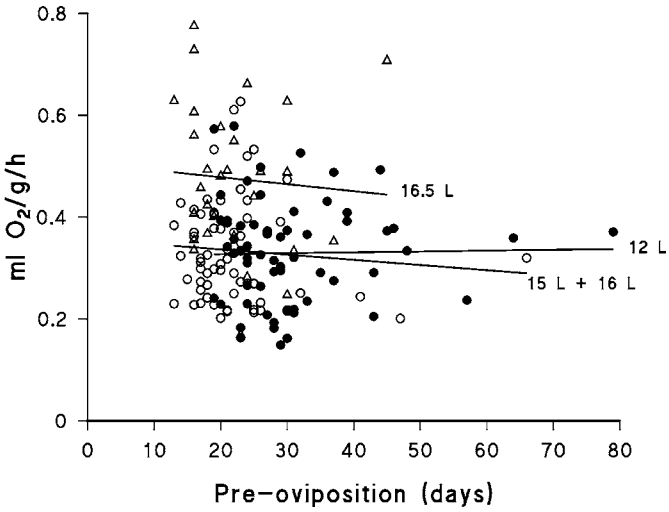

Fig. 6. Relationship between oxygen consumption and pre-oviposition period. Solid circles – 12L:12D, open circles – 15L:9D+16L:8D, open triangles – 16.5:7.5D. Only females in diapause are included. There was no correlation between oxygen consumption and duration of the pre-oviposition period (diapause intensity).

those that hatch later in the season are exposed to long days and develop more rapidly (Saunders 1983). This might help to keep the population more homogeneous phenologically and result in a more uniform photoperiodic induction of diapause and univoltinism (Saunders 1983). The population in the Czech Republic, however, is not uniformly univoltine (Hodek 1971b: Table 1, Honek & Sramkova 1976, Socha & Sula 1992). Short day-lengths (13.5L:10.5D) at the beginning of September and further decreasing would induce faster development in late summer and early autumn, so that the larvae are able to complete their development before the onset of winter, even those individuals that have tendency to bivoltinism. Lopatina et al. (2007) also suggest that the response of *P. apterus* to short day-lengths and low temperatures (below 24–25 °C) at the end of summer is to develop faster. That *P. apterus* takes longer to complete its development at intermediate day-lengths at 25 °C and a wider range of temperatures of 20–27 °C is confirmed,

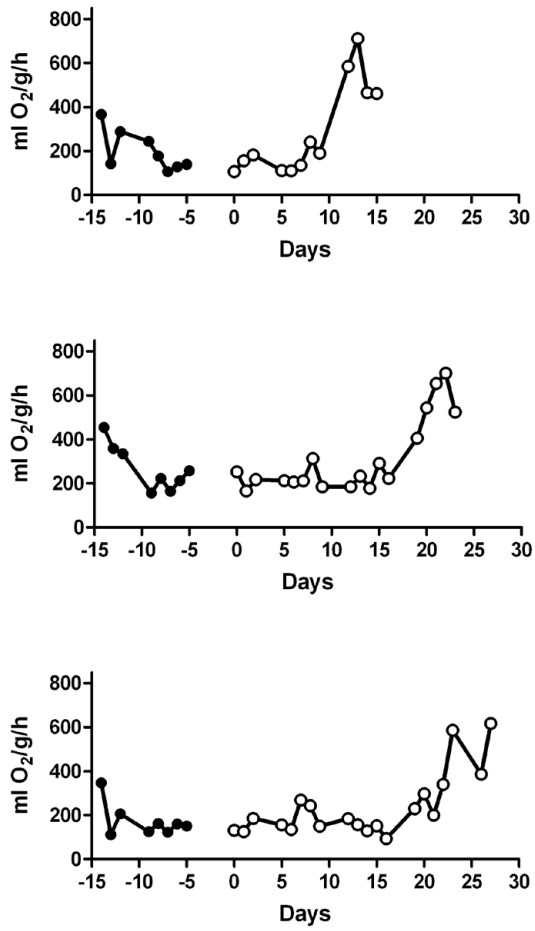

Fig. 7. Examples of oxygen consumption recorded during photoperiodic activation of diapausing individuals. Solid circles – oxygen consumption at 12L:12D, open circles – oxygen consumption at 18L:6D. Females were transferred from 12L:12D to 18L:6D on day 0 and their oxygen consumption measured until they started laying eggs (the end of the curve).

respectively, by Saunders (1983) and Numata et al. (1993). This explanation is in accordance with the results of Kidokoro & Masaki (1978), who report a similar decrease in developmental rate near the critical photoperiod for induction of embryonic diapause in both uni- and bivoltine populations of *Dianemobius nigrofasciatus*, and assume that this enables this species to enter diapause at the right time. Similarly a low growth rate at photoperiods near the critical daylength is also reported for the pentatomid bug *Dolycoris baccarum* (Nakamura 2003).

(*2*) *Chronobiological phenomenon*
The photoperiodic counters in each species of insect need a specific number of diapause promoting days for the induction of diapause (Saunders 2002). Slow development of *P. apterus* at the critical photoperiod may enable it to accumulate a sufficient number of photoperiodic cycles for induction. This explanation is supported by the greater tendency of the individuals that develop slowly at the critical day length to enter diapause (Fig. 2). The possibility cannot be excluded, however, that the slow developing individuals have a greater genetic tendency to enter diapause.

Relation between oxygen consumption and diapause intensity
In species like *P. apterus* short daylengths induce a deeper diapause than those around the critical level (Fig. 4). There is as yet little information on the physiological pathways leading to various levels of diapause intensity. It appears that the difference in the intensity of diapause recorded at 12L:12D and 16L:8D is not connected with metabolic rate, measured in terms of oxygen consumption (Fig. 5).

Previously we found that oxygen consumption measured at 25 °C of *P. apterus* in deep diapause that was induced in a 25/15 °C thermoperiod was greater than those in diapause induced at a constant 25 °C (Kalushkov et al. 2001). In addition, individuals that develop under a thermoperiod are also bigger than those that develop at a constant temperature (Novakova & Nedved 1999). To eliminate a possible effect of the change in temperature, we used a constant temperature of 25 °C for both rearing the insects and measuring their oxygen consumption. The present results are not consistent with reports for two lepidopteran species, for which oxygen consumption is considered to be a good indicator of diapause intensity (Varjas & Saringer 1998).

During the course of diapause development at 15 °C and a 18L:6D photoperiod in both *P. apterus* and *Aelia acuminata* there is a slight increase in oxygen consumption from November to April and decrease in diapause intensity, indicated by a shortening of the pre-oviposition period (Hodek & Hodkova 1981). In the present experiments, however, the oxygen consumption of females transferred from 12L:12D to an activation photoperiod of 18L:6D did not increase until the ovaries started to develop (Fig. 7).

Although a temperature around 15 °C is not suitable for photoperiodic activation, it does result in slow diapause development (Hodkova & Hodek 1987). Therefore, it is plausible that the start of morphogenesis occurred after a long period between November and April at 15 °C and this is responsible for the slight increase in oxygen consumption. In *Osmia lignaria*, oxygen consumption at 22 °C after spending approximately the first 100 days of their diapause development at either 0, 4 or 7 °C (i.e. lower than 15 °C), started to diverge only after termination of diapause (Sgolastra et al. 2010) .

Overall, neither diapause induction by different daylengths (Fig. 5) or diapause activation by long days (Fig. 7) are associated with the level of oxygen consumption. Diapause intensity is apparently due to a qualitative difference in gene expression (Denlinger 2002) that does not affect their metabolic rate. We did, however, record big differences in the oxygen consumption of diapause and non-diapause (reproducing) females (Fig. 5), as previously recorded in *P. apterus* (Slama 1964, Kalushkov et al. 2001).

CONCLUSIONS

(1) Duration of the pre-oviposition period of 1.5–2 month old females activated by different inductive photoperiods, when diapause is deepest, differed.
(2) Larval development is longest at intermediate photoperiods with a tendency for that of non-diapause individuals to be shorter. This may be related to either the synchronization of development or chronobiological mechanisms.
(3) If we accept that the duration of the pre-oviposition period of activated females is a criterion of diapause intensity, then oxygen consumption and diapause intensity are mutually independent. Oxygen consumption is low and similar in females aged 1.5-2 months irrespective of the inductive photoperiod. In addition, repeated measurements of the oxygen consumption of females during activation, indicates that the start of the increase in consumption is associated with the maturation of oocytes and not a decrease in diapause intensity.

REFERENCES

Beck S. D. 1980: *Insect Photoperiodism*. *Second Edition*. New York: Academic Press, 387 pp.

Bell R. A., Rasul G. G. & Joachim F. G. 1975: Photoperiodic induction of the pupal diapause in the tobacco hornworm, Manduca sexta. *Journal of Insect Physiology* **21**:1471–1480.

Canard M. 1983: La sensibilité photopériodique des larves de la chrysope Nineta flava. *Entomologia Experimentalis et Applicata* **34**: 111–118.

Danks H. V. 1987: *Insect Dormancy: An Ecological Perspective*. Ottawa: Biological Survey of Canada, 439 pp.

Denlinger D. L. 2002: Regulation of diapause. *Annual Review of Entomology* **47**: 93122.

Goryshin N. I. & Volkovich T. A. 1984: The role of graduality factor in the induction of the photoperiodic reaction in Pyrrhocoris apterus. *Zoologičeskij Žurnal* **63**: 1181–1191.

Havelka Y. 1980: [Some aspects of the photoperiodism of the carnivorous gall midge Aphidoletes aphidimyza (Diptera, Cecidomyiidae)]. *Entomologičeskoe Obozrenie* **59**: 241–248 (in Russian) [English translation in *Entomological Review* **59**: 1–8].

Hodek I. 1968: Diapause in females of Pyrrhocoris apterus L. (Heteroptera). *Acta Entomologica Bohemoslovaca* **65**: 422–435.

Hodek I. 1971a: Sensitivity of larvae to photoperiods controlling the adult diapause of two insects. *Journal of Insect Physiology* **17**: 205–216.

Hodek I. 1971b: Termination of adult diapause in Pyrrhocoris apterus (Heteroptera: Pyrrhocoridae) in the field. *Entomologia Experimentalis et Applicata* **14**: 212–222.

Hodek I. 1978: Role of temperature in diapause of Pyrrhocoris apterus (Heteroptera). *Věstník Československé Společnosti Zoologické* **42**: 172–187.

Hodek I. 1983: Role of environmental factors and endogenous mechanisms in the seasonality of reproduction in insects diapausing as adults. Pp.: 9–33. In: Brown V. K. & Hodek I. (eds): *Diapause and Life Cycle Strategies in Insects.* The Hague: W. Junk, 283 pp.

Hodek I. 2002: Controversial aspects of diapause development. *European Journal of Entomology* **99**: 163–173.

Hodek I. 2012: Diapause/Dormancy. Pp.: 275–342. In: Hodek I., van Emden H. F. & Honek A. (eds): *Ecology and Behaviour of the Ladybird Beetles* (*Coccinellidae*). Chichester, UK: Wiley-Blackwell, 561 pp.

Hodek I. & Hodková M. 1981: Relationship between respiratory rate and diapause intensity in adult of two heteropteran species. *Věstník Československé Společnosti Zoologické* **45**: 28–35.

Hodkova M. & Hodek I. 1987: Photoperiodic summation is temperature-dependent in Pyrrhocoris apterus (L.) (Heteroptera). *Experientia* **43**: 454–456.

Hodková M., Okuda T. & Wagner R. M. 2001: Regulation of corpora allata in females of Pyrrhocoris apterus (Heteroptera) (a mini-review). *In vitro Cellular and Developmental Biology – Animal* **37**: 560–563.

Honěk A. 1983: Rate of larval development and imaginal diapause in Pyrrhocoris apteruss. *Entomologia Experimentalis et Applicata* **34**: 90–95.

Honěk A. & Šrámková K. 1976: Behavioral regulation of developmental cycle in Pyrrhocoris apterus L. (Heteroptera: Pyrrhocoridae). *Oecologia* **24**: 277–281.

Kalushkov P., Hodková M., Nedvěd O. & Hodek I. 2001: Effect of thermoperiod on diapause intensity in Pyrrhocoris apterus (Heteroptera Pyrrhocoridae). *Journal of Insect Physiology* **47**: 55–61.

Kidokoro T. & Masaki S. 1978: Photoperiodic response in relation to variable voltinism in the ground cricket, Pteronemobius fascipes Walker (Orthoptera: Gryllidae). *Japanese Journal of Ecology* **28**: 291–298.
Kimura M. T. 1990: Quantitative response to photoperiod during reproductive diapause in the Drosophila auraria species-complex. *Journal of Insect Physiology* **36**: 147–152.
Kostal V., Tollarova M. & Dolezel D. 2008: Dynamism in physiology and gene transcription during reproductive diapause in a heteropteran bug, Pyrrhocoris apterus. *Journal of Insect Physiology* **54**: 77–88.
Lopatina E. B., Balashov S. V. & Kipyatkov V. E. 2007: First demonstration of the influence of photoperiod on the thermal requirements for development in insects and in particular the linden-bug, Pyrrhocoris apterus (Heteroptera: Pyrrhocoridae). *European Journal of Entomology* **104**: 23–31.
Masaki S. 2002: Ecophysiological consequences of variability in diapause intensity. *European Journal of Entomology* **99**: 143–154.
Musolin D. R. & Numata H. 2003: Photoperiodic and temperature control of diapause induction and colour change in the southern green stink bug Nezara viridula. *Physiological Entomology* **28**: 65–74.
Nakamura K. 2003: Effect of photoperiod on development and growth in a pentatomid bug, Dolycoris baccarum. *Entomological Science* **6**: 11–16.
Nakamura K. & Numata H. 2000: Photoperiodic control of the intensity of diapause and diapause development in the bean bug, Riptortus clavatus (Heteroptera: Alydidae). *European Journal of Entomology* **97**: 19–23.
Novakova J. & Nedved O. 1999: Developmental time and body mass in Pyrrhocoris apterus (Heteroptera, Pyrrhocoridae) under contrasting photo- and thermoperiods. *Entomological Problems* **30**: 97–100.
Numata H. & Hidaka T. 1983: Photoperiodic control of adult diapause in the bean bug, Riptortus clavatus Thurnberg (Heteroptera: Coreidae). II. Termination of diapause induced under different photoperiods. *Applied Entomology and Zoology* **18**: 439–441.
Numata H., Saulich A. H. & Volkovich T. A. 1993: Photoperiodic responses of the linden bug, Pyrrhocoris apterus, under conditions of constant temperature and under thermoperiodic conditions. *Zoological Science* **10**: 521–527.
Saunders D. S. 1983: A diapause induction-termination assymetry in the photoperiodic responses of the linden bug, Pyrrhocoris apterus and an effect of near-critical photoperiods on development. *Journal of Insect Physiology* **29**: 399–405.
Saunders D. S. 2002: *Insect Clocks*. *Second Edition*. Amsterdam: Elsevier, 560 pp.
Sgolastra F., Bosch J., Molowny-Horas R., Maini S. & Kemp W. P. 2010: Effect of temperature regime on diapause intensity in an adult-wintering Hymenopteran with obligate diapause. *Journal of Insect Physiology* **56**: 185–194.
Sláma K. 1964: Hormonal control of respiratory metabolism during growth, reproduction, and diapause in female adults of Pyrrhocoris apterus L. (Hemiptera). *Journal of Insect Physiology* **10**: 283–303.
Socha R. & Šula J. 1992: Voltinism and seasonal changes in haemolymph protein pattern of Pyrrhocoris apterus (Heteroptera: Pyrrhocoridae) in relation to diapause. *Physiological Entomology* **17**: 370–376.
Spieth H. R. & Sauer K. P. 1991: Quantitative measurement of photoperiods and its significance for the induction of diapause in Pieris brassicae (Lepidoptera, Pieridae). *Journal of Insect Physiology* **37**: 231–238.
Tauber M. J. & Tauber C. A. 1972: Geographic variation in critical photoperiod and in diapause intensity of Chrysopa carnea (Neuroptera). *Journal of Insect Physiology* **18**: 25–30.
Tauber M. J., Tauber C. A. & Masaki S. 1986: *Seasonal Adaptations in Insects*. New York: Oxford University Press, 411 pp.
Tauber M. J. & Tauber C. A. 2015: Phenological responses of Pseudomallada (Neuroptera: Chrysopidae): Comparative data from three Nearctic species and interspecific hybrids. *European Journal of Entomology* **112**: 49–62.
Varjas L. & Såringer G. 1998: Oxygen consumption as an indicator of diapause intensity in pupae of Lacanobia oleracea and Mamestra brassicae reared at different inductive photoperiods. *Acta Phytopathologica et Entomologica Hungarica* **33**: 147–151.