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# Influence of pre-diapause temperature on intensity of summer and winter diapause in the cabbage butterfly *Pieris melete* (Lepidoptera: Pieridae)

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**Key words.** *Pieris melete*, temperature, photoperiod, aestivation, hibernation, diapause intensity

**Abstract.** Effect of pre-diapause temperature on summer and winter diapause intensity was examined under both laboratory and field conditions. Under short photoperiods of 8L : 16D and 10L : 14D, all pupae entered diapause at 15, 18 and 20°C and the incidence of diapause dropped to 82.3% and 85.5% at 22°C, respectively. Under long photoperiods of 14L : 10D and 16L : 8D, the incidence of diapause decreased with increasing temperature and there were significant differences among temperatures. The incidence of diapause at 16L : 8D was significantly lower than that under14L : 10D at 20 and 22°C. By transferring diapause pupae induced under various temperatures (18, 20 and 22°C) at a short day of 10L : 14D or a long day of 14L : 10D, to 12.5L : 11.5D, 20°C, the duration of summer diapause induced under 22°C (mean 76.1 days) was significantly shorter than those under 20°C (mean 85.9 days) and 18°C (mean 90.9 days), showing that the incidence of summer diapause was positively linked to the intensity of summer diapause; whereas the duration of winter diapause induced under 10L : 14D was similar at 18°C (89.2 days), 20°C (88.7 days) and 22°C (89.2 days) and there were no significant differences. Field experiments also showed that the high rearing temperatures significantly decreased the incidence and intensity of summer diapause, but had no significant affect on the intensity of winter diapause. When the naturally aestivating pupae from the first spring generation (formed on 24 April) and second spring generation (formed on 15 May) were kept under summer conditions, the diapause duration of the first generation lasted for 107–166 days (mean 146 days), about twenty days longer than that of the second generation [lasted for 92–151 days (mean 126 days)]. All results reveal that the sensitivity to temperature prior to aestivation and hibernation was quite different.

## INTRODUCTION

Photoperiod and temperature influence the intensity of diapause in a number of different species. These environmental factors are experienced by the pre-diapause growth stages, but their influence is expressed in the relative intensity of the ensuing diapause (Beck, 1980; Tauber et al., 1986; Danks, 1987; Koštál, 2006). Influence of temperature during diapause induction on the intensity of diapause could vary between species but also among individuals of the same species. In some species, high temperatures during diapause induction induce more intense diapause than low temperatures (Masaki, 1962, the cricket *Teleogryllus emma*; Sieber & Benz, 1980, codling moth *Cydia pomonella*; Denlinger & Bradfield, 1981, tobacco hornworm *Manduca sexta*; Beck, 1989, corn borer *Ostrinia nubilalis*; Wellso, 1991, Hessian fly *Mayetiola destructor*; Roditakis & Karandinos, 2001, grape berry moth *Lobesia botrana*; Kalushkov et al., 2001, Nedvěd & Vambera, 2002, bug *Pyrrhocoris apterus*; Asano et al., 2004, yellow-spotted longicorn beetle, *Psacothea hilaris*). In others, diapause was more intense after rearing at a low temperature (Cox, 1975, the warehouse moth *Ephestia calidella*; Bell 1976, Indian meal moth *Plodia interpunctella*; Principi et al., 1990, chrysopid *Mallada flavifrons*; Glitho et al., 1991, bruchid *Bruchidius atrolineatus*; Fantinou et al., 2003, corn stalk borer *Sesamia nonagrioides*). In *Mamestra brassicae*, the summer diapause, induced under long days, is shorter than the short-day-induced winter diapause, but its intensity is increased by high temperatures during induction (Masaki & Sakai, 1965).

The cabbage butterfly, *Pieris melete* Ménétriés is multivoltine with a pupal summer and winter diapause. In the field, there are two distinct infestation peaks per year, one in spring of one generation (In some years a small proportion of the population that pupates in mid or late April can emerge and may produce a second generation) and a second in autumn of one to three generations (Xue et al., 1996). The photoperiodic response curves of *P. melete* showed an intermediate response type (Xue et al., 1997, Fig. 1). The short daylengths (< 12 h) induced 100% winter diapause regardless of temperature. The intermediate daylengths (12 to 13.25 h) induced most pupae to develop without diapause. The long daylengths (≥ 13.5 h) induced summer diapause and the incidence of diapause was obviously affected by both photoperiod and temperature. Influence of pre-diapause photoperiod on diapause intensity in *P. melete* was also investigated (Table 3, in Xiao et al., 2006). The results show that the duration of diapause is significantly influenced by the pre-diapause photoperiod. At 20°C the durations of diapause induced by photoperiods of 12L : 12D (65.9 d), 10L : 14D (69.7 d), or 8L : 16D (68.1 d) were significantly shorter than at 13L : 11D, (94.5 d), 14L : 10D (102.8 d), or 16L : 8D (80.6 d). There were no significant differences in the intensity of diapause induced by short photoperiods of

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TABLE 1. A comparison of diapause incidence and duration of diapause (mean ± SE) in *P. melete* when the different generations of larvae hatched at different times were reared under natural conditions. Diapausing pupae were kept at 12.5L : 11.5D, 20°C to terminate diapause.

| | Day-length[1] | Temperature[2] (°C) | No. of pupae observed | Diapause (%) | Duration of diapause[3] (n) |
|---|---|---|---|---|---|
| SG1 | 12 h 57 min – 13 h 49 min | 18.6 | 5720 | 98.13 | 93.24 ± 1.45 (136) a |
| SG2 | 14 h 05 min – 14 h 43 min | 21.2 | 1211 | 94.55 | 86.70 ± 1.32 (118) b |
| SG3 | 14 h 43 min – 14 h 56 min | 25.0 | 154 | 10.38 | 70.07 ± 4.59 (14) c |
| AG1 | 13 h 22 min – 12 h 30 min | 24.3 | 757 | 1.59 | — |
| AG2 | 12 h 30 min – 11 h 59 min | 21.9 | 721 | 89.60 | 94.24 ± 1.49 (119) a |
| AG3 | 11 h 43 min – 11 h 11 min | 14.6 | 738 | 100 | 90.64 ± 1.51 (140) a |

[1] Natural day-length from day of hatching to pupation (including twilight); [2] the mean natural temperature from the day of hatching to pupation; [3] values followed by different letters are significantly different based on one-way analysis of variance (ANOVA) and a multiple Bonferroni test.

12L : 12D, 10L : 14D and 8L : 16D, whereas differences induced by the long photoperiods of 13L : 11D, 14L : 10D and 16L : 8D were significantly different.

So far, the influence of pre-diapause temperature on the intensity of summer diapause has been investigated only in a few insects. However, *P. melete* is an excellent animal for studying the influence of pre-diapause temperature on diapause intensity, as summer diapause is highly sensitive to temperature. It is therefore of interest to examine whether pre-diapause temperature has a significant influence on diapause intensity.

## MATERIAL AND METHODS

In the experiments we used specimens collected from the suburbs of Nanchang (28°46′N, 115°50′E, about 50 m a.s.l.), Jiangxi Province, P.R. China. Full-grown larvae prior to pupation were collected from wild crucifers in late November 2005 and transferred to wooden insectaries (30 × 30 × 35 cm) to pupate and overwinter under natural condition. Adults emerged in spring 2006 and were transferred to an outdoor screened insectary for mating and oviposition. The eggs were collected, placed in incubators and the newly hatched larvae were reared in round plastic boxes (7.5 × 6 cm) each containing fresh Chinese cabbage (*B. juncae* var. *communis*) leaves and at least 50 larvae. When the larvae reached the fifth instar, they were transferred to two boxes to pupate.

In order to determine whether diapause incidence is linked to diapause intensity, the incidence of diapause in larvae reared under two short photoperiods of 8L : 16D and 10L : 14D (for the induction of winter diapause) and two long photoperiods of 14L : 10D and 16L : 8D (for the induction of summer diapause) at 15, 18, 20 and 22°C until they pupated, was recorded. All treatments were repeated three times. Then the diapausing pupae induced by the short photoperiod of 10L : 14D or long photoperiod of 14L : 10D at 18, 20 and 22°C were transferred to 12.5L : 11.5D, 20°C to terminate diapause. The criterion of diapause termination was adult emergence; and the duration of diapause included the period of post-diapause development.

To examine the effects of seasonal variations on the incidence and duration of summer and winter diapause, the adults from the overwintering pupae were released into an outdoor screened insectary for reproduction. From this insectary we obtained 107 non-diapausing pupae from the 5720 pupae of the first spring generation (SG1). We successfully reared another two successive spring generations, using these non-diapausing pupae, in this outdoor screened insectary under natural conditions and obtained three different generations of aestivating pupae. The first spring generation that pupated on 24 April (as SG1), second generation on 15 May (as SG2) and third generation on 15 June (as SG3) were selected for diapause termination (Table 1). On the tenth day after pupation, those aestivating pupae were divided into two groups. In one group, aestivating pupae from SG1, SG2 and SG3 were transferred to round plastic boxes and kept under a diapause-preventing photoperiod of 12.5L : 11.5D at 20°C to terminate diapause. In other group, aestivating pupae from SG1 and SG2 were maintained under natural conditions to terminate diapause.

In autumn, we also successfully reared three successive autumn generations (as AG1, the first autumn generation, AG2 and AG3, respectively, Table 1) by using the adults from aestivating pupae in this outdoor screened insectary under natural conditions. As a few individuals entered diapause in AG1, only the diapausing pupae from AG2 and AG3 were used to record the termination of diapause at 12.5L : 11.5D, 20°C.

Laboratory experiments were performed in illuminated incubators (LRH-250-GSII) with adjustable temperature and photoperiod. The incubators were equipped with four fluorescent 30 W tubes controlled by an automatic time switch. Light intensity at the level of the insects was about 700 lx and the variation in temperature was ± 0.5°C.

Statistical analyses were conducted using the STATA package Version 9.0. Bonferroni test (*P* = 0.05) was used to

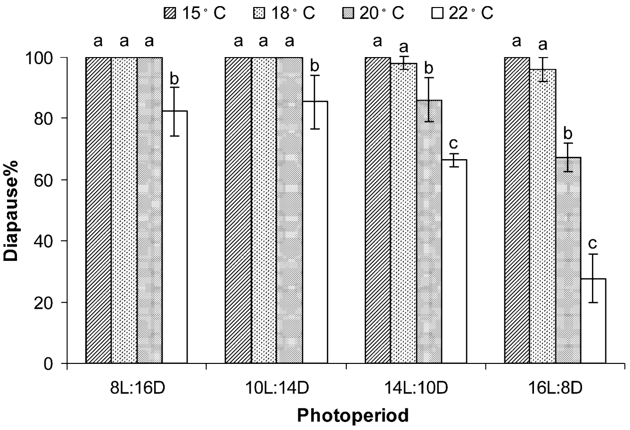

Fig. 1. Diapause incidence of aestivation and hibernation induced at different photoperiods and constant temperatures. Values followed by different letters are significantly different based on Bonferroni test and one-way analysis of variance (ANOVA) (F = 14.75, d.f. = 3, 8, *P* = 0.0013 for 8L : 16D, F = 8.44, d.f. = 3, 8, *P* = 0.0073 for 10L : 14D, F = 36.93, d.f. = 3, 8, *P* = 0.0000 for 14L : 10D and F = 137.56, d.f. = 3, 8, *P* = 0.0000 for 16L : 8D). n = 47–167 for each treatment.

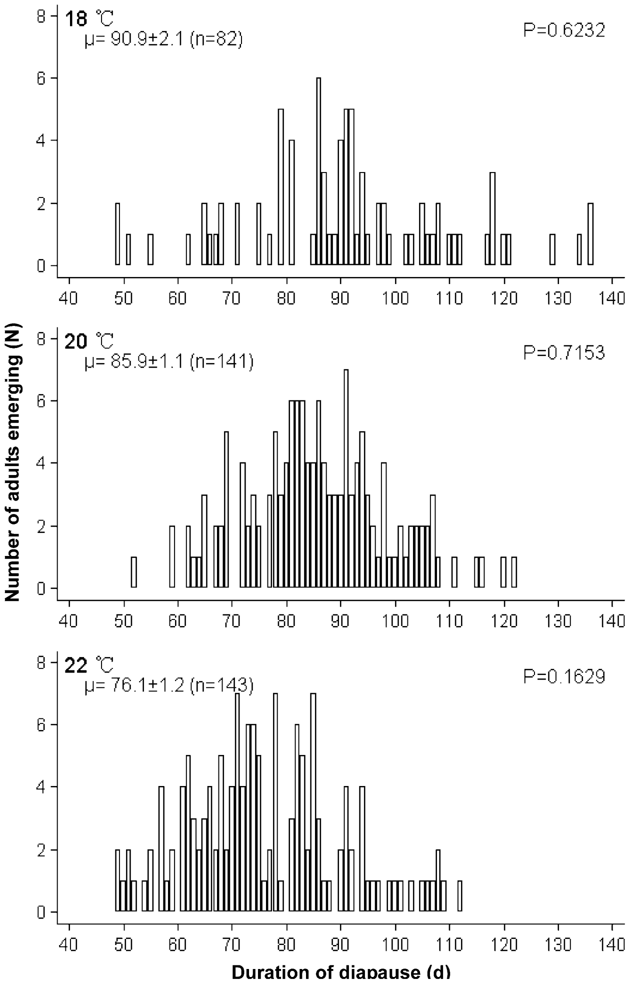

Fig. 2. Duration of summer diapause at 12.5L : 11.5D, 20°C in *P. melete*. Diapause was induced under 14L : 10D, at 18°C, 20°C and 22°C.

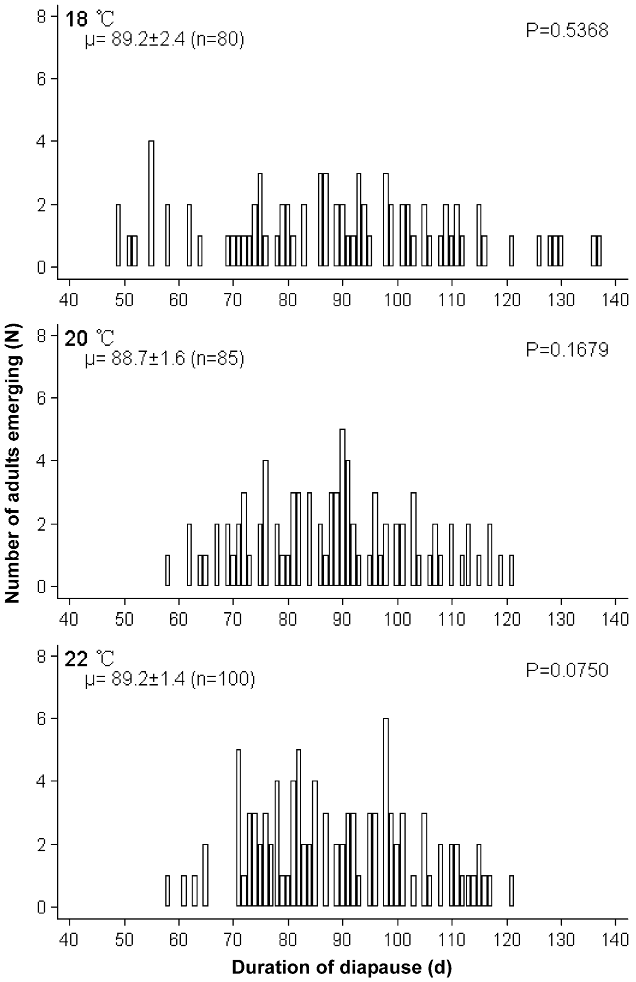

Fig. 3. Duration of winter diapause at 12.5L : 11.5D, 20°C in *P. melete*. Diapause was induced under 10L : 14D, at 18°C, 20°C and 22°C.

determine whether the variance in the incidence of diapause in the different temperature and photoperiodic regimes treatment were significant. Date for the duration of diapause was tested for normality using Skewness/Kurtosis tests. One-way analysis of variance (ANOVA) and Bonferroni multiple comparison at *P* = 0.05 was used to determine whether differences in the duration of diapause among different generations and treatments were significant.

## RESULTS

### Effect of temperature on the induction of summer and winter diapause

Effect of temperature on the incidence of diapause was quite different for winter and summer diapause (Fig. 1). Under short photoperiods of 8L : 16D and 10L : 14D, all pupae entered diapause at 15, 18 and 20°C. The incidence of diapause dropped to 82.3% under 8L: 16D and 85.5% under 10L: 14D at 22°C, but there was no significant difference between these two treatments [Bonferroni test: *P* (= 0.6564) > 0.05]. However, under long photoperiods of 14L : 10D and 16L : 8D, the incidence of diapause decreased with increasing temperature (100% at 15 and 18°C, 86.2% at 20°C, and 66.4% at 22°C under 14L : 10D; 100% at 15°C, 95.9% at 18°C, 67.3% at 20°C, and 27.8% at 22°C under 16L: 8D) and there were significant difference among temperatures [F = 36.93, d.f. = 3,8, *P* (= 0.0000) < 0.01 for 14L : 10D and F = 137.56, d.f. = 3,8, *P* (= 0.0000) < 0.01 for 16L : 8D). Statistical analyses also showed that the incidence of diapause at 16L : 8D was significantly lower than under 14L : 10D at 20 and 22°C (Bonferroni test: *P* = 0.008 at 20°C, *P* = 0.022 at 22°C).

### Effect of pre-diapause temperature on summer and winter diapause intensity

The differences among the durations of pupal diapause induced under various temperatures (18, 20 and 22°C) at a short day of 10L : 14D or a long day of 14L : 10D were examined at 20°C, 12.5L : 11.5D (Fig. 2 and Fig. 3). The duration of summer diapause induced by 22°C (mean 76.1 ± 1.2 days) was significantly shorter than those by 20°C (mean 85.9 ± 1.1 days) and 18°C (mean 90.9 ± 2.1 days) (F = 29.00, d.f. = 2, 363, *P* (= 0.0000) < 0.01; Bonferroni test: *P* = 0.000 between 20 as well as 18°C and 22°C, *P* = 0.049 between 18°C and 20°C) (Fig. 2). However, the duration of winter diapause induced under 10L : 14D was similar at 18°C (89.2 ± 1.4 days), 20°C (88.7 ± 1.6 days) and 22°C (89.2 ± 2.4 days), and there were no

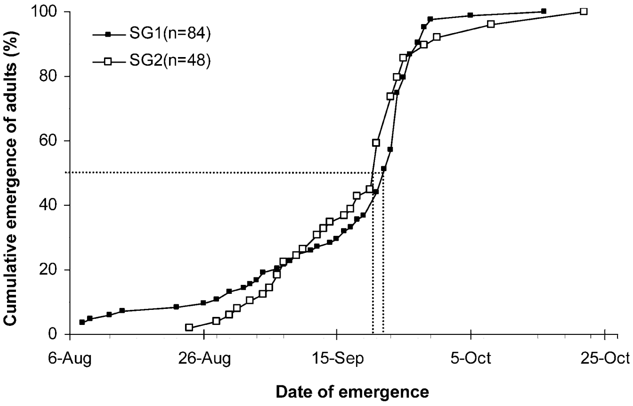

Fig. 4. Diapause termination of aestivating pupae from the first spring generation (formed on 24 April) and second spring generation (formed on 15 May) under natural conditions.

significant differences (F = 0.02, d.f. = 2, 262, *P* (= 0.9795) > 0.05; Bonferroni test: *P* = 1.000 between all of the three treatments) (Fig. 3). These results indicate that high rearing temperature decreased the intensity of summer diapause, but did not significantly influence the intensity of winter diapause.

## The incidence of summer and winter diapause in three successive spring and autumn generations under natural conditions and their diapause termination at 12.5L : 11.5D and 20°C

Table 1 shows the effects of naturally changing photoperiods and temperatures on diapause incidence and subsequent diapause intensity. In spring generations, the incidence of diapause as well as diapause intensity gradually declined with increasing daylengths and temperatures. The incidence of diapause in three successive spring generations was 98.13%, 94.55% and 10.38%, respectively. The duration of diapause induced under 25.0°C (70.1 d) was significantly shorter than that at 21.2°C (86.7 d) or at 18.6°C (93.2 d) (F = 15.97, d.f. = 2, 265, *P* (= 0.0000) < 0.01; Bonferroni test: *P* = 0.001 between 25.0°C and 21.2°C and *P* = 0.000 between 25.0°C and 18.6°C). In autumn generations, percentage of diapause gradually increased as the daylengths and temperatures decreased. In AG1, only 1.59% individuals entered diapause when they were exposed to intermediate daylengths (13 h 22 min to 12 h 30 min) and a high mean temperature of 24.3°C during mid to late September, the incidence of diapause in the second and third autumn generations, was increased to 89.60% and 100%, respectively. However, there were no significant differences in diapause intensity between AG2 and AG3 (F = 2.86, d.f. = 1,257, *P* (= 0.092) > 0.05), although the rearing temperature was quite different between AG2 (21.9°C) and AG3 (14.6°C). These results further indicate that high rearing temperatures decrease the intensity of summer diapause, but did not significantly influence the intensity of winter diapause.

## Duration of summer diapause under natural conditions

When the naturally aestivating pupae from the first spring generation (formed on 24 April, diapause induced under the daylengths from 12 h 57 min to 13 h 49 min and 18.6°C) and second generation (formed on 15 May, diapause induced under the daylengths from 14 h 5 min to 14 h 43 min and 21.2°C) were kept under summer conditions, the diapause duration of the first generation lasted for 107–166 days (mean 146 days), about twenty days longer than that of the second generation [lasted for 92–151 days (mean 126 days)]. However, the period of adult emergence from both groups of aestivating pupae was almost synchronized. The date of emergence of 50% of the individuals occurred between 21 (for SG2) and 22 (SG1) September for aestivating pupae of both generations (Fig. 4).

## DISCUSSION

High temperature is generally reported to enhance induction of summer diapause (Masaki, 1980; Tauber et al., 1986; Danks, 1987). However, high temperature strongly weakened the diapause-inducing effects of long daylengths in *P. melete* and resulted in a low incidence of diapause (Fig. 1). Furthermore, the incidence of summer diapause was positively linked to the intensity of summer diapause. The lower the incidence of diapause, the shorter the duration of diapause (Fig. 2, Table 1).

The influence of pre-diapause temperature on winter diapause intensity/duration has been studied in a number of insects (see Introduction), but there are few studies on summer diapause. The intensity of the pupal summer diapause in the noctuids *M. brassicae* and *M. oleracea*, is increased by rearing the larvae under long days at a high temperature (Masaki & Sakai, 1965; Poitout & Bues 1977). In the ten-spotted lady beetle *Epilachna admirabilis*, rearing temperatures of 20–30°C did not affect the intensity of summer diapause (Imai, 2004). In the zygaenid moth, *Pseudopidorus fasciata*, the duration of prepupal summer diapause induced under 25 and 28°C is similar at 25°C, 13L : 11D (Wu et al., 2006, Table 2). Unlike the above species, the duration of summer diapause in *P. melete* decreased with increasing temperature in both the laboratory (Fig. 2) and under natural conditions (Table 1). The high rearing temperatures significantly decreased diapause intensity compared to relatively low temperatures. Such a mechanism may serve to synchronize emergence between the early diapause and later-diapause individuals in autumn. Field observations support this (Fig. 4). The aestivating pupae formed on 24 April underwent a long summer diapause (mean 146 days), whereas those formed on 15 May underwent a short diapause (mean 126 days). However, adults from both mainly emerged during the same period, end of August to mid-October.

As diapause incidence is mostly linked to diapause intensity, one would expect that the action of low temperatures during induction of winter diapause would strengthen diapause. In *E. calidella*, diapause is more intense when induced (by short days) at 20°C rather than 30°C (Cox, 1975). In *M. flavifrons*, the 3rd instar larvae have a diapause twice as long after induction in the 2nd instar at 15°C than at 25°C (Principi et al., 1990). In *S. nonagrioides*, the number of days needed for 50% of diapausing larvae to pupate is much less when the larvae are reared at 25°C than at 20°C (Fantinou et al., 2003). However, pre-diapause temperature did not affect the intensity of winter diapause in *P. melete*. The duration of the different hibernating generations was almost the same (AG2, 94 days and AG3, 91 days) (Table 1). According to our recent experiments, the rate of diapause development during winter diapause was strongly affected by low temperature during diapause. Low temperature obviously enhanced the development and termination of winter diapause. For example, when the naturally induced hibernating pupae were exposed to a low temperature of 5°C for 60 days and then transferred to 12.5L : 11.5D, 20°C to terminate diapause, the mean diapause duration (75.3 days) was significantly shorter than that (94.2 days) when they were kept under 12.5L : 11.5D, 20°C without prior exposure to a temperature of 5°C.

All our results indicate that the sensitivity to temperature prior to aestivation and hibernation is quite different in *P. melete*.

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