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Diapause development in *Aquarius paludum* (Heteroptera: Gerridae)

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Abstract. Diapause fixation and development were studied in females of a Czech population of *Aquarius paludum* by monitoring changes in oxygen uptake, weight and reproduction potential in field adults between August and February. The fall in oxygen uptake related to fresh weight (from > 1000 µl O_2 per g per h to around 500 µl O_2 per g per h) in field adults during early diapause was similar in two age cohorts, although the time of adult ecdysis differed by 50 days and thus occurred at markedly different temperatures and photoperiods of late August vs. early October. The different conditions affected the weight of females and thus also the absolute value of oxygen consumption: both parameters were much lower in the October females. The seasonal time of diapause termination in *A. paludum* did not differ from findings in other cold temperate insects: diapause and the photoperiodic response ended in the winter, as was shown by the possibility of insects' reactivation by 26°C in spite of a diapause promoting daylength of 12L : 12D. In most females (78.6%) ovaries matured after transfer to these conditions in mid-February, while ovarian maturation occurred in only 16.7% of females transferred in early January and no ovarian maturation was observed in females transferred in early December. Ovarian maturation was preceded by a transient increase in oxygen consumption from 600 µl O_2 per g per h to 1400 µl O_2 per g per h.

INTRODUCTION

Although life-history adaptations and diapause induction under various environmental conditions were studied in several water strider species of the genus *Aquarius* (Heteroptera: Gerridae) (Blanckenhorn, 1994; Blanckenhorn & Fairbairn, 1995; Harada, 1994, 1998; Inoue & Harada, 1997; Harada et al., 2000; Harada & Spence, 2000), the condition of the bugs in the course of diapause development and its termination remained relatively neglected, except for *A. paludum*. Gradual increase in daylength after winter solstice within the range of short days promoting diapause accelerated diapause development: strong positive phototaxis appeared early in diapause adults of *A. paludum* under long days, when they had previously experienced the increase in photophase within short-day photoperiods. The positive phototaxis appeared 2 weeks earlier than when they were kept only under constant short days before the transfer to long days (Harada, 1991). In the Kochi (33°N) population of *Gerris gracilicornis* which is univoltine and adopts obligatory diapause, both short days in fall and the subsequent winter chilling were needed to end the very long diapause lasting more than 7 months (Harada & Taniguchi, 2001). In other Gerridae the evidence on the physiological parameters of insects in diapause is very poor. The course of diapause termination (Hodek, 1971, 1979, 1983) and changes in oxygen consumption (Hodek & Hodkova, 1981) were, however, studied in detail in some terrestrial bugs, e.g. in *Pyrrhocoris apterus* (Pyrrhocoridae) or *Aelia acuminata* (Pentatomidae). The aim of this preliminary study was to check the eventual differences between aquatic and terrestrial heteropterans, particularly to establish the time of diapause termination.

MATERIAL AND METHODS

Insects

Adults and last instar larvae of *Aquarius paludum* (Heteroptera: Gerridae) were collected in ponds of south Bohemia (near to Ceske Budejovice, 49°N) during late August and September of 2000. Insects were reared outdoors in containers with water and floating pieces of polystyren until December. Adult females aged 3–4 months were subsequently transferred to either 26 ± 1°C and short-day photoperiod (12L : 12D) on 7 December or 5 ± 2°C and continuous darkness in containers with moist moss on 26 December. We believed that the latter conditions adequately substituted for hibernation shelters which have not yet been found in nature. Females were stored under the latter conditions until 8 January or 12 February when they were transferred to 26 ± 1°C and short-day photoperiod (12L : 12D). Oxygen consumption was measured every 2nd or 3rd day after the transfer of females to 26°C. At the end of each experiment, females were dissected under Ringer saline for insects and the state of ovaries was monitored. The state of testes is not a suitable indication of the progress of diapause development. Changes in oxygen consumption during 2–3 months after adult ecdysis were measured in early (ecdysis on 20 August) and late (ecdysis on 5 October) females kept continuously outdoors. Outdoor temperatures are given in Fig. 3. Insects kept outdoors or at 26°C were fed with frozen adult flies (*Sarcophaga bullata*).

Oxygen consumption

The metabolic rate was measured as oxygen consumption manometrically with a Warburg respirometer at 26°C (Slama, 1960). Respiratory vessels of about 10 ml volume were employed with 5% potassium hydroxide to absorb carbon dioxide and with a small piece of wet cotton to maintain constant pressure of water vapor. The recorded value of oxygen consumption of each individual at rest represented an average from three 0.5 h readings. Females were weighed using an analytical balance (Sartorius) with the accuracy of 0.1 mg.

Statistics

Means ± SEM are given in Figures and in the text. Difference between means was evaluated by Student's T-test.

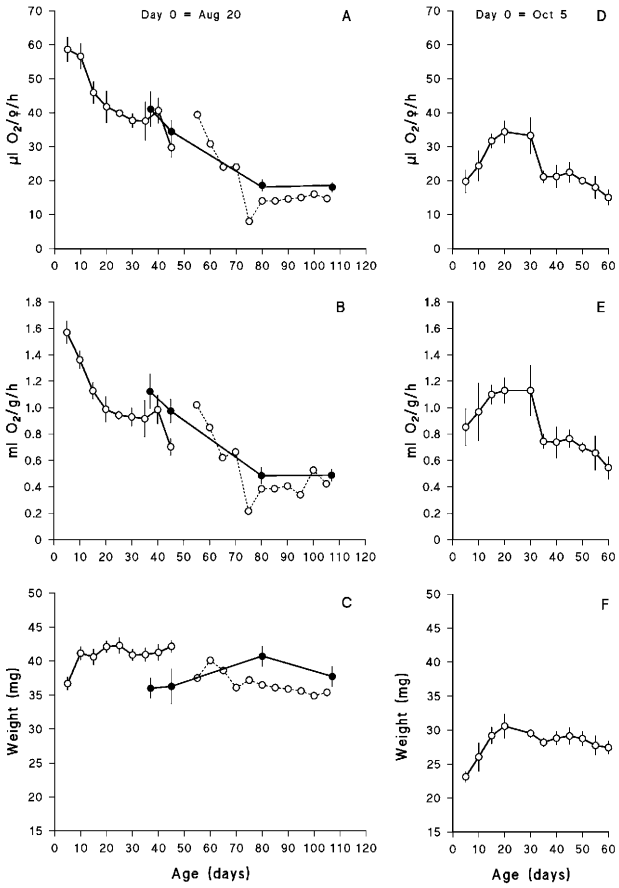

Fig. 1. Changes in oxygen consumption and fresh weight in two age cohorts of *A. paludum* females under field conditions. Day 0 = the day of adult ecdysis. Open circles, means ± SEM for every five days (full line, N = 5 for August females, N = 3 for October females, dotted line, N = 1). Closed circles, means ± SEM for females measured at corresponding dates but the age is not precisely defined (N = 10).

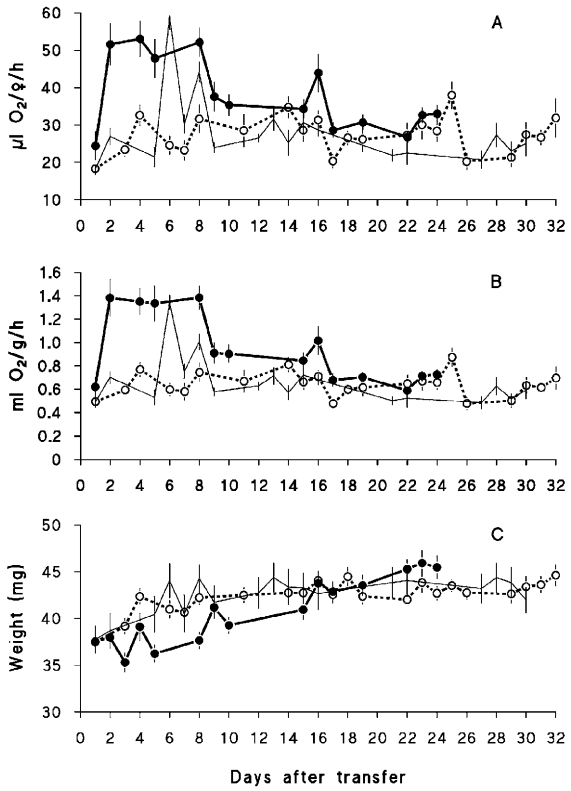

Fig. 2. Changes in oxygen consumption and fresh weight in *A. paludum* females transferred to 26 ± 1°C and 12L : 12D. Day 1 = the day of transfer to 26 ± 1°C from field conditions on 7 December (thin line, N = 10), from 5 ± 2°C on 8 January (open circles, N = 12–15) or 12 February (closed circles, N = 14–15). Two last samples were transferred from the field to 5 ± 2°C on 26 December. Means ± SEM are given. Difference between means of oxygen consumption (A, B, days 2–8) for January and February samples is significant (P << 0.0001) (Student's T-test).

RESULTS

Oxygen consumption in the field

The adults that ecdysed in late August differed substantially from those ecdysed in early October, both in absolute oxygen consumption and weight. The freshly ecdysed August bugs consumed around 60 µl O_2 per female per h, while the October individuals only about 20 µl O_2 (Fig. 1A, D). The average for the first 50 days of adult age was 42.6 ± 1.8 µl O_2 for the August females and 24.5 ± 1.7 µl O_2 for October females (P << 0.0001). When the oxygen consumption is related to the fresh weight, the difference becomes smaller (Fig. 1B, E), because also the weight differed much between the two samples (Fig. 1C, F). The August females consumed 1043.5 ± 47.2 µl O_2 per g per h, while the October females 890.4 ± 60.7 µl O_2 per g per h (P < 0.05). In both cohorts the oxygen consumption gradually decreased to the level of around 500 µl O_2 per g per h in females aged >50 days (Fig. 1B, E). The starting weight of August females was about 35 mg and it was only about 23 mg in October females. In both cohorts the weight increased during the first 10–20 days of life, so that the average value for the first 50 days was 40.9 ± 0.4 mg and 27.6 ± 0.6 mg in August and October, respectively (P << 0.0001).

Transfers to laboratory

The changes in diapause intensity during diapause development were estimated by incubations of three winter samples in a high temperature of 26 ± 1°C and a diapause promoting photoperiod of 12L : 12D. One sample was directly transferred from outdoor to incubation conditions on 7 December. Two samples from 26 December were first exposed to 5 ± 2°C for 2 and 6 weeks, so that they were transferred to incubation conditions on 8 January and 12 February, respectively. An important progress in diapause development evidently occurred between the last two dates, as was shown both by changes in oxygen consumption and weight during a 4 week incubation and by the state of ovaries after a 4 week incubation. The oxygen consumption in the January females remained near the level of 500–700 µl O_2 per g per h (18–35 µl O_2 per female per h) during the one month experiment, while the oxygen consumption in the February females sharply increased within one day to 1400 µl O_2 per g per h (50 µl O_2 per female per h) and was maintained at this level for at least one week (Fig. 2A, B). The weight of February females was lower during the first week but gradually increased during the next 3 weeks of incubation to about 47 mg. The weight of January females remained at a similar level during the 4 week incubation (Fig. 2C). Much higher incidence of females with matured eggs was recorded in February females (78.6%, N = 14) than in January females (16.7%, N = 12). Ovaries of all December females (N = 10) remained in pre-vitellogenic state.

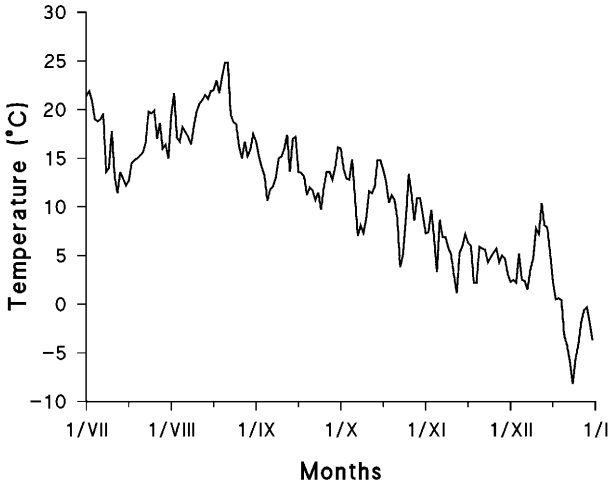

Fig. 3. Daily averages of field temperatures during the relevant period.

DISCUSSION

Physiological conditions of field bugs

The oxygen consumption per fresh weight is related to the age of adult females rather than to the date of sampling and outdoor conditions. This is shown by comparison of two age cohorts of females of *A. paludum* on the same date. On 10 October, the early ecdysed August females were 50 days old, while the late ecdysed October females were only 5 days old. While the oxygen consumption on that date already decreased to the values around 700 µl O_2 per g per h in the August females due to diapause induction (Fig. 1B), the oxygen consumption in the young October females was increasing (Fig. 1E). In spite of changed environmental conditions (daylength decreased by about 2 h and the average daily temperature by about 6°C, Fig. 3), October females aged 15 days consumed more than 1000 µl O_2 per g per h and thus showed similar metabolic levels as the young August females aged 15 days (Fig. 1B, E). During the pre-diapause, the young bugs have to feed to accumulate energetic reserves in order to survive the starvation of the long hibernation period. At the age of 50 days both groups of females had similar oxygen consumption of around 700 µl O_2 per g per h. Oxygen consumption further decreased to the level of around 500 µl O_2 per g per h, evidently typical for diapause. This value is similar to the values found in diapausing terrestrial bugs: in *Dolycoris baccarum* 480 µl O_2 per g per h (Conradi-Larsen & Sømme, 1978) and in *Pyrrhocoris apterus* 500 µl O_2 per g per h (Hodek & Hodkova, 1981). Also, similar, but double values were ascertained for active females in both terrestrial and aquatic bugs.

Progress of diapause development

Diapause intensity in *A. paludum*, indicated by several characteristics after the transfer from outdoors or 5 ± 2°C to 26 ± 2°C and 12L : 12D, did not decrease substantially between early December and early January, but it did before early February. This was indicated by the ability of February females to increase abruptly the oxygen consumption after the transfer (Fig. 2B). The increased food consumption led to the increase in weight (Fig. 2C) and a resumption of ovarian maturation in 78.6% of females, in spite of exposure to diapause promoting short daylength. The fall in oxygen consumption after about one week incubation at 26°C might be due to temperature compensation of metabolism which is well documented in ectothermic organisms (Precht et al., 1973). Alternatively, the metabolic cost of the transition from non-reproductive to reproductive state may be higher than the maintenance of reproduction. The time of diapause termination in *A. paludum* does not differ from the general picture for adult diapause in insects in the cold moderate climate (Tauber et al., 1986; Danks, 1987). Similar time of diapause end was found in a terrestrial heteropteran, *P. apterus* (Hodek, 1971).

Geographical difference in the role of temperature on termination of diapause

Diapause of the Kochi population in relatively weak. It was terminated quite easily by transfer to high temperature of 25°C, irrespective of the previous duration of diapause induced in larvae by short day photoperiod (12L : 12D, 20°C) (Harada & Taneda, 1989). In the population from Ceske Budejovice (49°N), in the middle of diapause, i.e. in December or January, diapause was not terminated by transfer to 26°C. Their diapause was induced in field conditions and the bugs were then kept at 5°C before transfer. It cannot be excluded that the difference between Japanese and Czech populations was partly caused by different diapause inducing conditions.

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