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Influence of temperature acclimation on respiration-temperature relationship in *Tetrodontophora bielanensis* (Collembola: Onychiuridae)

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Collembola, *Tetrodontophora bielanensis*, respiration rate, temperature, acclimation

Abstract. CO_2 output in *Tetrodontophora bielanensis* was measured in the temperature range 0 to 20°C using gas chromatography after acclimation to 5, 13 and 19°C in the laboratory. The effect of acclimation temperature on mean CO_2 output was not significant. The relationship between respiration and experimental temperature was found to vary depending on the acclimation temperature. It was not possible to obtain a significant mathematical description of the relationship after acclimation to 5°C. The relationship was exponential at acclimation temperature 13°C and linear after acclimation to 19°C. The importance of acclimation to temperature and metabolic cold adaptation in different populations is discussed.

INTRODUCTION

Ecophysiological studies dealing with respiration – temperature relationships in small arthropods are often focused on the differences between species living in Arctic, Antarctic and alpine areas, and between temperate and lowland habitats (Zinkler, 1966; Block & Young, 1978; Strømme et al., 1986; Klekowski & Opalinski, 1989; Sømme, 1989; Sømme et al., 1989; Strømme, 1989). The elevated rate of oxygen consumption observed in some cold-adapted species is explained mainly in terms of metabolic compensation in the sense of Krogh (1916). The concept of metabolic cold adaptation has been criticized recently by Clarke (1991). Sømme (1989) suggested that experiments be conducted on the same or closely-related species within the same mass range and occupying similar ecological niches by using identical techniques to enable more accurate comparisons between alpine and lowland Collembola respiration rates. Several studies dealing with intraspecific variation in the respiration rate of arthropods have been published [Nylund (1991) and Schultz et al. (1992) for beetles, Zettel (1985) and Šustr & Šimek (1994) for springtails].

The influence of laboratory acclimation to different temperatures on respiration rate was investigated by Woude & Joosse (1988) in two species of springtails in an attempt to explain seasonal differences in oxygen consumption. Differences between alpine and lowland populations, as well as seasonal changes in the shape of the CO_2 output rate – temperature relationship were observed in *Tetrodontophora bielanensis* Waga (Collembola) by Šustr & Šimek (1994).

In this study the influence of laboratory acclimation to 5, 13 and 19°C on the CO_2 output rate and CO_2 production – temperature relationship was studied in *T. bielanensis* to explain the role of temperature acclimation.

MATERIAL AND METHODS

T. bielanensis is a large stenothermic and hygrophilic collembolan. The experimental animals were collected in November 1992 from a deciduous forest between limestone rocks on a south-east slope in Říčka Valley near Brno, Czech Republic, at 300 m a.s.l. The animals were active in moss on the rocks, and in the lower layers of litter.

After transport to the laboratory and storage in darkness at 10°C for 24 h the animals were subjected to acclimation temperatures (AT) 5, 13 and 19°C for 44 days. Collembola were maintained in plastic boxes (3.5 × 3.5 cm, 5 individuals per box) at the acclimation temperature (± 0.5°C) in darkness. High atmospheric moisture was ensured by placing the boxes in a large PVC container with moist plaster of Paris on the bottom. Mortality and moulting rates were measured during acclimation. Mortality was expressed as a percentage of the initial number of animals in each experimental group. The exuviae were collected at 3 day intervals and moulting rate expressed as the number of exuviae per animal. The presence and duration of egg laying were observed.

After acclimation, CO_2 production was measured at experimental temperatures (ET) 0, 5, 10, 15 and 20°C (Fig. 1). The live body mass of the measured animals ranged from 1.11 to 17.16 mg (mean ± S.E.: 5.54 ± 0.22).

For respiration measurements, Plastipak syringes (Becton Dickinson, France, cat. no. 23G1") were used as incubation vessels. A small piece of plastic tube permitted only a small movement of the plunger (0.2–1.0 ml) within the syringe, thus protecting the specimen from being crushed. For determination of the respiration rate a small piece (5 × 5 mm) of filter paper saturated with distilled water to maintain a high relative humidity, was placed in the syringe together with one animal. The plunger was immediately inserted and adjusted to 0.85 ml, the syringe needle blocked by insertion into a rubber stopper, and the time noted. Then syringes with the test animals (and the empty controls with the same filter paper but without animals) were placed in a water bath and incubated at the appropriate temperature in darkness. After 2 h incubation, 0.6 ml of the air in the syringe was injected directly into a gas chromatograph.

Analyses were performed with a HP 5890A gas chromatograph (Hewlett-Packard, USA) equipped with a thermal conductivity detector and fitted with a 80–100 mesh Porapak Q column (Šimek & Šustr, 1995). The column was operated at 45°C using He as a carrier gas (the flow rate was 0.5 $ml.s^{-1}$). The temperature of the injector and detector was 65°C. The peak areas were measured using a HP 3393A

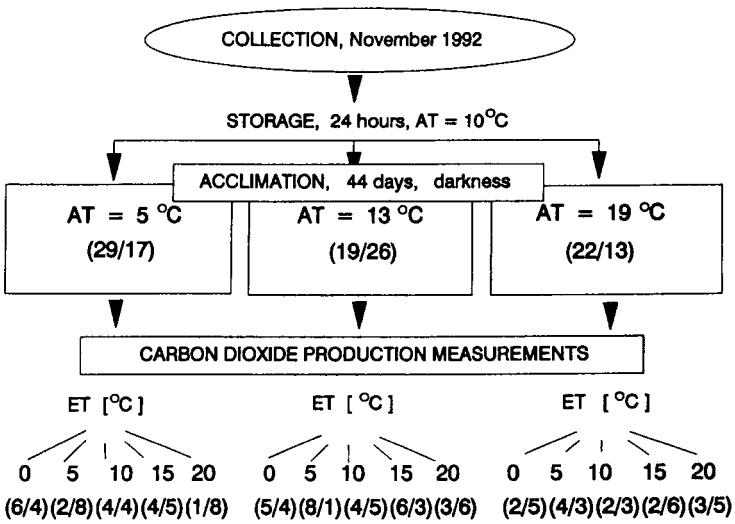

Fig. 1. The scheme of the experimental design. Number of replications in brackets (females/males). AT – acclimation temperature; ET – experimental temperature.

integrator. A single analysis took approximately 4 min. For calibration purposes an injection was made using a known volume of a standard gas mixture, containing 1% of CO_2 and 20% of O_2 in N_2 (Scott Specialty Gases, USA, obtained from Supelco, Switzerland, cat. No. 2-3441). The results were expressed as the amount of carbon dioxide in each syringe after incubation compared with the amount of carbon dioxide in control syringes taken as equivalent to CO_2 in ambient air. The experimental animals were weighed after the measurements.

The differences between the slopes of regression lines were tested by T-test. To quantify the difference in the shapes of the CO_2 production-temperature curves the linearity of the relationships between M/W^b and ET (linear regression) as well as log M/W^b and ET (exponential regression) were tested by ANOVA with regression (Weber, 1972) (M is respiration rate in µl $CO_2.h^{-1}$, W is fresh body mass in g and b is the slope of the regression line between log M and log W). M/W^b (size-adjusted CO_2 output rate in µl $CO_2.g^{-b}.h^{-1}$) is independent on body mass. This expression of metabolic rate was used by Studier & Lavoie (1990) and Šustr & Šimek (1994) to compare respiration rate of animals of different body mass. In this paper the expression is used to give values comparable with those in Šustr & Šimek (1994).

RESULTS

The mortality rates during the 44 days acclimation were relatively low in groups acclimated to 5 and 13°C (3.2 and 3.7% of initial numbers, respectively). It was somewhat higher (19.6%) in the group acclimated to 19°C, but the difference between the slopes of regression lines of number of dead animals per individuum on time was not significant ($P > 0.05$). No eggs were deposited by this latter group during acclimation. In the 5°C group, the first egg was observed after 8 days and eggs were deposited throughout the following 20 days. The first eggs appeared later (11th day) at 13°C and the oviposition period was

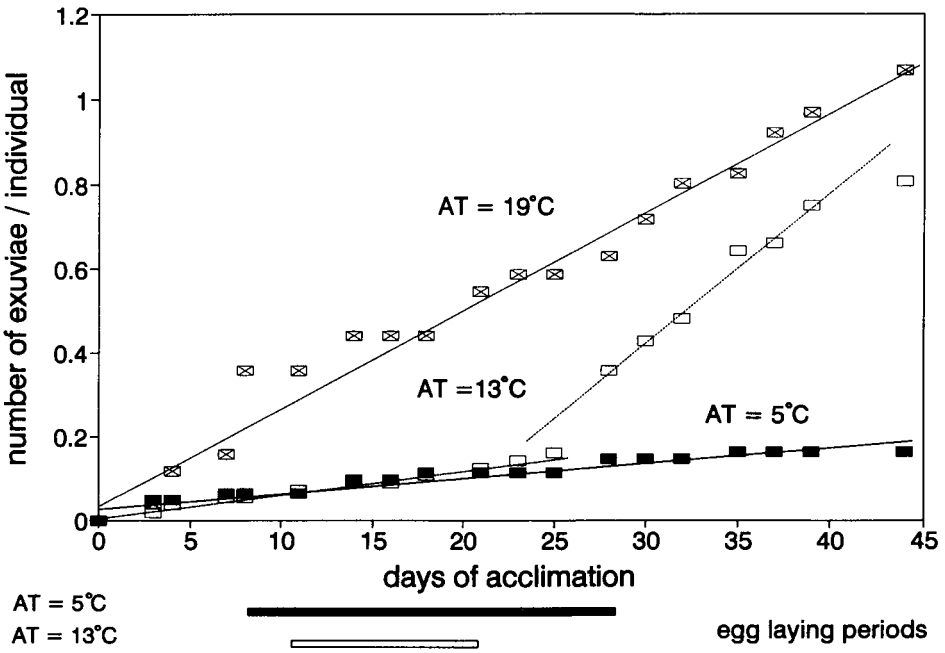

Fig. 2. The influence of acclimation to three temperatures on moulting rate and egg laying of *Tetrodontophora bielanensis*. Differences between the slopes of the regression lines were significant ($P < 0.05$). AT as in Fig. 1. Egg laying was not observed at AT = 19°C.

shorter (10 days) in this group. The moulting rate was dependent on temperature as well as on oviposition. At 19°C the moulting rate was high during the entire acclimation period, but it was very low during egg deposition at 5 and 13°C. After egg laying had finished the moulting rate increased rapidly in animals acclimated to 13°C in contrast to cold-acclimated ones (Fig. 2).

The mean (± S.E.) live body mass of animals after the experiments was 6.55 ± 0.31 mg (n = 73) in females and 4.25 ± 0.18 mg (n = 57) in males. The difference between the sexes was significant ($P < 0.01$). The mean body mass of animals acclimated to 5, 13 and 19°C was 5.51 ± 0.25 mg (n = 47), 5.18 ± 0.33 mg (n = 47) and 6.05 ± 0.57 mg (n = 36), respectively. The influence of acclimation temperature on fresh body mass was not significant ($P > 0.1$). The linear regression of log CO_2 output rate (μl $CO_2.h^{-1}$) on log fresh mass (g) was calculated for each experimental group separately. The slopes of the regression lines (b) did not differ significantly ($P > 0.1$) and they lie in the range from 0.21 to 1.80 (number of replications from 5 to 10, r from 0.22 to 0.95). The common value of the slope (b = 1.03 ± 0.08) differed significantly from zero ($P > 0.1$, r = 0.76). This value was taken for computing size-adjusted CO_2 output rate (SAR) (μl $CO_2.g^{-b}.h^{-1}$) in this study.

The SAR was similar in both sexes ($P > 0.1$, Table 1). The effect of acclimation temperature was not significant ($P > 0.1$, Table 1). Statistical analysis based on unbalanced two way ANOVA revealed significant differences in SAR due to ET ($P < 0.01$, Table 2).

TABLE 1. Size-adjusted CO_2 output rate (SAR) of *Tetrodontophora bielanensis* depending on sex and acclimation temperature (AT) (the results of the unbalanced two way ANOVA). In μl $CO_2.g^{-b}.h^{-1}$ (mean ± S.E.).

	AT (°C)			
	5	13	19	All AT's
Females	73.20 ± 6.74	81.08 ± 14.42	66.74 ± 9.12	73.31 ± 5.55
	n = 29	n = 19	n = 22	n = 70
Males	78.29 ± 13.03	52.40 ± 4.56	53.57 ± 7.52	60.54 ± 4.98
	n = 17	n = 26	n = 13	n = 56
Total	75.08 ± 6.34	64.51 ± 6.88	61.85 ± 6.40	
	n = 46	n = 45	n = 35	

TABLE 2. Differences in size-adjusted CO_2 output rate (SAR) of *T. bielanensis* due to experimental temperature (ET). The mean SAR pooled for all acclimation temperatures. In μl $CO_2.g^{-b}.h^{-1}$ (mean ± S.E.).

ET (°C)				
0	5	10	15	20
26.57 ± 2.17	42.55 ± 2.92	73.45 ± 5.44	92.01 ± 7.25	104.55 ± 9.75
n = 26	n = 26	n = 22	n = 26	n = 26

The differences in the shapes of the SAR – temperature relationships due to AT are evident from Fig. 3 (for Q_{10} see Table 3.). All relationships were approximately exponential in the experimental temperature range from 0 to 10°C. In animals acclimated to 5°C, the SAR increased from 10 to 15°C and decreased between 15 and 20°C. In the group acclimated to 13°C, the zone of relative temperature independence between 10 and 15°C and

rapid increase over 15°C was observed. The SAR increased very slowly from 10 to 20°C in the group acclimated to 19°C.

TABLE 3. The Q_{10} coefficients of size-adjusted CO_2 output rate (SAR) in *Tetrodontophora bielanensis* acclimated to different temperatures. AT and ET as in Fig. 1.

AT (°C)	ET range (°C)						
	0–5	0–10	5–10	5–15	10–15	10–20	15–20
5	2.42	2.89	3.45	2.60	1.95	1.13	0.65
13	2.24	2.28	2.34	1.72	1.26	1.92	2.92
19	3.55	3.49	3.44	2.16	1.36	1.31	1.27

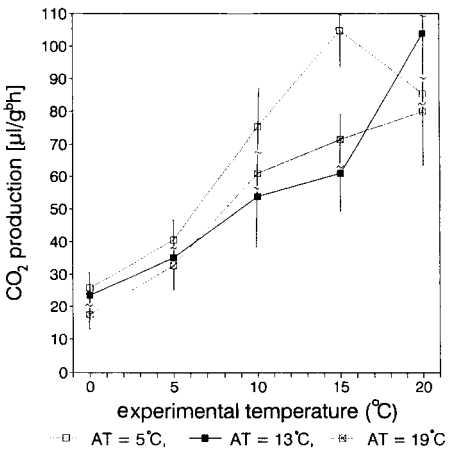

Fig. 3. The relationships between CO_2 production rate and experimental temperature in *Tetrodontophora bielanensis* acclimated to three different temperatures. AT as in Fig. 1. Error bars are standard errors.

The differences may be illustrated by means of the results of regression analysis in the range 0 to 20°C. It is not possible to obtain a significant mathematical description of the SAR – ET curve at 5°C by linear as well as exponential regressions ($P > 0.05$ in both cases), the best description of the relationship at AT = 13°C is given by the exponential curve ($P < 0.01$). The relationship is best fitted by a linear regression ($P < 0.01$) at AT = 19°C.

DISCUSSION

The higher mortality rate and the absence of oviposition at AT = 19°C indicated that the temperatures near to 20°C are above the normal temperature range of animals collected in late autumn. Monthly mean air temperature measured at the nearest meteorological station (Brno) decreased from 9.1°C in October to –0.5°C in December. Normal soil temperatures are between 0 and 20°C and temperatures between 5 and 10°C are preferred by many temperate species of springtails (Zinkler, 1966). The egg development in *T. bielanensis* with a long rest period during winter (Krzysztofowicz & Kisiel, 1986; Cassagnau, 1990) is adapted to oviposition in late autumn. Temperature decrease may play an important role in synchronization of oviposition.

The published data confirm the temperature dependence of moulting rate in springtails (Thibaud, 1977; Zettel, 1985). The mean duration of intermoults of non-laying animals acclimated to 19°C was 43 days (calculated from regression lines showed in Fig. 2.). The similar value (approximately 37 days) results from the relationship between intermoult duration and temperature showed by Thibaud (1977). The very low moulting rate at 5°C agree with an extrapolation of the Thibaud's data. However, the moulting rate decreased deep under the values reported by Thibaud (1977) during oviposition at 13°C. Further

studies are needed to explain the interactions between temperature, oviposition and moulting rate in this species.

A higher mean body mass was observed in females from the lowland population in June 1991. Such a mass difference between sexes was not significant in a mountain population (Šustr & Šimek, 1994). The influence of oviposition on the difference was discussed. However, the significant sexual differences were observed in autumn animals from lowland population after acclimation at all experimental temperatures in this study regardless of oviposition.

The common slope of the double logarithmic regression between CO_2 output rate and live mass (b = 1.03) is higher in comparison with the slope calculated previously (Šustr & Šimek, 1994) from pooled data for *T. bielanensis* collected in different seasons of the year in two different populations (b = 0.570). The explanation of this difference is not easy because of the large errors in the regression equations due to the narrow mass ranges and different experimental procedures used (long acclimation in this study). The changes of the slope during long periods in the laboratory were reported in a species of oribatid mite (Young, 1979). Zinkler (1966) reported b values near 1 for many collembolan species.

The elevation of SAR was confirmed only in animals from a lowland population collected in autumn, no influence of altitude on SAR being found by ANOVA (Šustr & Šimek, 1994). In the present study no changes in mean SAR due to acclimation temperature were observed using ANOVA. The differences in the shape of SAR-temperature relationship seem to be more important during temperature acclimation in *T. bielanensis*.

Woude & Joosse (1988) found no changes in the O_2 consumption of the collembolans *Orchesella cincta* and *Tomocerus minor* after experimental acclimation, when the data were analysed by ANOVA.

The difficulties encountered in interpreting the relationship between respiration and temperature are related to the complexity of respiration. Respiration is not a discrete process but a measure of demand for ATP, reflecting a variety of physiological processes (Clarke, 1991). Respiration in the respirometer may be significantly influenced by locomotory activity of animal. The simultaneous recording of locomotory activity of the measured animal is not possible in the majority of recent microrespirometric methods. Zinkler (1966) reported approximately 100% increase of oxygen consumption during maximal locomotion activity in *T. bielanensis* in the temperature range 5–18°C. The decrease of locomotion in the temperature range to which the animal is adapted may be one of the possible explanations for the zone of relative temperature independence – ZRTI (Precht et al., 1973; Newell et al., 1974). The ZRTI between 10 and 15°C was observed in *T. bielanensis* acclimated to 13°C. The inflection point at temperatures about 20°C in cold acclimated animals may be a result of stress caused by high experimental temperatures. The low sensitivity of SAR to temperature between 15 and 20°C in animals acclimated to 19°C may be due to the ZRTI being close to the temperature of acclimation or a consequence of stress after long acclimation at too high temperature.

The concept of cold metabolic temperature compensation presumes the elevation of the respiration rate at every experimental temperature, i.e. an upward shift of the respiration-temperature curve. However the present results correspond to left shift (downwards in temperature) of the curve. The CO_2 production rate was higher in animals acclimated to 5°C in the range 0–15°C but it was lower at 20°C in comparison to AT = 13°C. A similar

decrease in the respiration activity due to stress at too high ET may be expected at AT = 13°C, too (at ET's over 20°C). Zinkler (1966) interpreted the elevated oxygen consumption rate in the oligostenothermic collembolan *Isotoma hiemalis* in comparison to *Protaphorura armata* as the shift of the "optimal life range" of this species downwards in temperature rather than as a result of any compensation ("thermostable respiration"). The concept of metabolic cold adaptation was criticized by Clarke (1991) as an inappropriate approach to respiration as a discrete process. The changes of respiration are only a consequence of simultaneous changes of numerous processes. Therefore, the simple compensation of respiration rate to temperature as well as the selective advantage of increasing ATP production are questionable. However, the frequently observed elevation of respiration in terrestrial arthropods living in cold climates may be explainable in agreement with Clarke's objections. Terrestrial polar and alpine habitats are characterized by wide daily and seasonal variations of temperature (Sømme, 1989; Clarke, 1991). Many strategies of adaptation are possible. In *Isotoma hiemalis*, a higher level of standard metabolic rate at 0°C was observed in the individuals moving on the snow surface than in the animals remaining in litter (Zettel, 1985). However, elevated respiration rate was not observed in every case (see Nylund, 1991). The results obtained from investigations in isolated populations of the same species did not support the respiration elevation in mountain and high latitude populations unambiguously. Nylund (1991) reported higher oxygen consumption in a Netherlands population of the carabid *Calathus melanocephalus* in comparison to Norway populations, and respiration rate was elevated in 50% of all groups of *C. melanocephalus* after acclimation at low temperature. Schultz et al. (1992) found no differences in oxygen consumption between populations of the tiger beetle *Cicindela longilabris* from higher latitudes and populations occupying climatic refugia at lower altitudes. Šustr & Šimek (1994) observed a significant increase of size adjusted CO_2 production only within the same population, in animals collected in autumn in comparison with summer ones. No differences between lowland and mountain populations were observed.

In conclusion, the non-genetic adaptations resulting in changes of the shape of the respiration temperature relationship were confirmed in *T. bielanensis*. The changes may be interpreted as a consequence of the shift of the optimal life range downwards in temperature rather than as a result of any compensation.

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