

# Spiders on a treadmill: influence of running activity on metabolic rates in *Pardosa lugubris* (Araneae, Lycosidae) and *Marpissa muscosa* (Araneae, Salticidae)

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## Summary

The CO<sub>2</sub> release of the well-tracheated jumping spider, *Marpissa muscosa*, and the poorly tracheated, *Pardosa lugubris*, was tested while animals were running on a treadmill at three different speeds and under a selective elimination of lungs or tracheae. Thus, the influence of a well-developed tracheal system on the metabolism during physical exercise was examined. The CO<sub>2</sub> release in intact animals increased with the running speed in both species. The costs of transport (COT) running at the maximal sustainable speed were nearly twice as big in *M. muscosa* as in *P. lugubris*. Elimination of one lung by sealing resulted in reduced COT and running times, and

increasing anaerobic proportions in metabolism. Effects were greater in *P. lugubris* than in *M. muscosa*, indicating that tracheae compensate partly for the lacking lung capacity. Sealing of the tracheae in *M. muscosa* reduced the COT and the running times only at the highest speed. Results indicate that tracheae in *M. muscosa* support the aerobic metabolism only at the most intense physical exercise. At low and medium activity, tracheae may play their main role in the local supply of organs that are not involved in running activity.

Key words: respiration, tracheal system, book-lungs, Araneae, spider.

## Introduction

During the evolution of the araneomorph spiders, the second pair of book-lungs was completely reduced or was replaced by tracheae. In some spider families, tracheae are well-developed systems and often reach into the prosoma. The circumstances leading to the evolution of tracheae in spiders are still poorly understood. As one explanation, it was hypothesized that well-tracheated spiders have greater aerobic capabilities than the other spider families (Levi, 1967, 1976; Anderson, 1970).

For testing this hypothesis, comparative investigations of wolf and jumping spiders have been made (Prestwich, 1983a,b; Schmitz, 2004). Spiders of these two families have similar life styles and, additionally, they possess well-developed lungs with similar diffusing capacities (Schmitz and Perry, 2001, 2002). While wolf spiders only possess four simple tube tracheae that are restricted to the opisthosoma, jumping spiders have well-developed tracheae that reach into the prosoma and provide about 30% additional capacity for diffusive gas exchange *via* the walls of the entire tracheal system (Schmitz and Perry, 2001, 2002). Looking at the metabolic rates during maximum exercise, differences between wolf and jumping spiders are striking: Prestwich (1983b) found greater aerobic scopes, shorter recovery periods and smaller anaerobic dependence after maximum activity in a jumping spider (*Phidippus audax*) compared with a wolf spider (*Lycosa lenta*). Looking at the jumping spider *Marpissa muscosa* and the wolf

spider *Pardosa lugubris*, the maximum mass-specific CO<sub>2</sub> release and the factorial scopes during and after maximum activity were greater while recovery periods were shorter in the well-tracheated jumping spider (Schmitz, 2004).

Thus previous studies are consistent with the hypothesis that tracheated spiders have greater aerobic capabilities during exercise. Conversely, tracheae of jumping spiders do not directly supply the prosomal muscles (Schmitz and Perry, 2000). The direct oxygen supply *via* terminal diffusion from the tracheal endings into the muscles, which would be the most effective pathway, is therefore not possible (Schmitz and Perry, 2000). Thus, tracheae could increase oxygen delivery to the muscles only by gas exchange over the walls of the entire tracheal system and *via* an oxygen transport by the haemolymph. But in jumping spiders most tracheae run bundled through the petiolus into the prosoma where they mostly end in the nervous system and the gut epithelium. This reduces the lateral diffusing capacity of the entire tracheal system (diffusive conductance of the tracheal walls) by about 20% (Schmitz and Perry, 2001). For these reasons, the role of the tracheae of jumping spiders in gas exchange during exercise is still unclear and it can be hypothesized that tracheae alone are not responsible for the greater aerobic capabilities in this spider group. This hypothesis was tested in the present study by measuring the CO<sub>2</sub> release of *M. muscosa* and *P.*

*lugubris* during constant running on a treadmill and under selective elimination of respiratory organs. The elimination of the tracheae or of one lung by sealing in comparison with intact animals should reveal the role of tracheae and lungs in gas exchange during physical exercise in differently tracheated spiders.

## Materials and methods

### Animals

Females and males of *P. lugubris* L. and *M. muscosa* Clerck were collected in the vicinity of Bonn and were maintained individually in plastic containers at room temperature (19–21°C). Animals were fed *Drosophila spec.* twice per week, while water was constantly available. Animals were fed 2–3 days before the experiment, thus being in a post-absorptive state during the measurements.

### Respirometry – treadmill

An open-flow system was used to measure rates of CO<sub>2</sub> release during activity on a miniature airtight custom-made treadmill. Animals moved in the horizontal plane in an experimental chamber that was an upright oriented cylinder of about 3 cm<sup>3</sup>. At the bottom of the chamber, an axle-driven rubber treadmill belt was moved by a step-less motor support, which could vary the speed of the treadmill between 0 and 8 cm s<sup>-1</sup>. Outside air was pumped through the system with a flow rate of 100 ml min<sup>-1</sup>, adjusted by an Aalborg flow meter (Oranienburg, NY, USA). The air initially passed through a series of containers filled with NaOH and a Soda lime scrubbing column to remove both CO<sub>2</sub> and water. Air was then rehydrated by a saturated NaCl solution to 60% r.h., passed through the reference chamber of the gas analyser and the animal chamber, and was finally drawn into the CO<sub>2</sub>-analyser (URAS 14, ABB; ABB Process Industries GmbH, Frankfurt, Germany). The CO<sub>2</sub> analyser interfaced with a PC for data-acquisition; the sampling rate was 1 sample per second.  $\dot{V}_{\text{CO}_2}$  (mass specific CO<sub>2</sub> release per time) was calculated from fractional concentrations of CO<sub>2</sub> entering (*FI*) and leaving (*FE*) the animal chamber using the equation (from Withers, 1977):

$$\dot{V}_{\text{CO}_2} = (FE_{\text{CO}_2} - FI_{\text{CO}_2}) \times \text{flow rate},$$

where flow rate is 100 ml min<sup>-1</sup> and *FI* is zero. The rate of CO<sub>2</sub> release was converted to amount of CO<sub>2</sub> per unit time and gram body mass (nmol s<sup>-1</sup> g<sup>-1</sup>) at STPD. Volumes of the experimental chamber and of the connecting tubes caused time lags of 10 s between the experimental chamber and the CO<sub>2</sub> analyser. This time lag was tested with a defined amount of CO<sub>2</sub> that was blown into the animal chamber. All times given in the results, including Figs 1 and 2 were already corrected for these values. Animals were weighed before and after each set of experiments and the average mass during a single experiment was estimated assuming a linear mass decrease over the time.

Animals were tested at a constant temperature of 20°C at three different speeds. As *P. lugubris* has longer legs than *M.*

*muscosa*, these speeds differed between the species. The lowest speed was that speed at which animals just walked in a constant manner (fast walking; 1.45 cm s<sup>-1</sup> in *P. lugubris* and 1.0 cm s<sup>-1</sup> in *M. muscosa*). The medium speed was a slow running (2.3 and 1.8 cm s<sup>-1</sup>, respectively). The highest speed was the maximum speed that animals could sustain for at least five minutes (fast running; 3.35 and 2.5 cm s<sup>-1</sup>, respectively). The final speed of each run was adjusted within the first 10 s of the experiment. Animals were watched during the entire run and the treadmill was stopped as soon as the animal stopped and tried to hold fast to any part of the animal chamber.

All experiments started with the lowest speed and ended with the highest one, to accustom animals to the treadmill and to the enforced running. Between the runs at least 2 h of recovery were inserted. Individuals were tested with unimpaired respiratory organs (intact animals) and if possible the same animals were tested with eliminated tracheae or with one eliminated lung. As not all animals could be tested in all states, the numbers of tested animals were not the same for all measurements (Tables 1–3, Figs 3–5). The single spiracle of the tracheal system or one of the both spiracles of the lungs was sealed the day before the experiment. We used the rubber cement Fixogum (© Marabu; GmbH & Co. KG, Tamm, Germany) for sealing, which could be removed after the experiment without injuring the animals. Animals were first anaesthetised with CO<sub>2</sub> and then fixed to a soft plate with strips of plasticine. After animals were awake and the respiratory organs were allowed to refill with fresh air, the respective spiracles were glued, after which animals were released. Some animals were tested twice with intact respiratory organs to test for individual differences between different days.

The steady state  $\dot{V}_{\text{CO}_2}$  (nmol s<sup>-1</sup> g<sup>-1</sup>) during treadmill running was calculated from the individual CO<sub>2</sub> release traces. It was determined as mean value of the recorded data in periods when CO<sub>2</sub> release did no longer increase or decrease. Minimal costs of transport (*C*<sub>min</sub>, nmol cm<sup>-1</sup> g<sup>-1</sup>) were determined by regression of the steady state CO<sub>2</sub> release during treadmill running to the speed and by calculating the mean slope of all individual regression lines. The costs of transport (COT, nmol cm<sup>-1</sup> g<sup>-1</sup>) were calculated from the steady state values divided by the speed. As not all animals reached steady state CO<sub>2</sub> release at all speeds, another evaluation with the data was carried out that calculates the CO<sub>2</sub> release per distance (nmol cm<sup>-1</sup> g<sup>-1</sup>) and the metabolic rate ( $\dot{V}_{\text{CO}_2}$ , nmol s<sup>-1</sup> g<sup>-1</sup>) using the amount of CO<sub>2</sub> released during the period of running. Differences between animal groups were tested using ANCOVA statistics.

## Results

Most individuals of both species behaved well on the treadmill and run constantly at the three speeds. The few animals that refused to run and tried to hold fast to the walls of the animal chamber from the beginning were eliminated from the experiments. Differences between males and females in both species in the metabolic rate did not exist (ANCOVA,

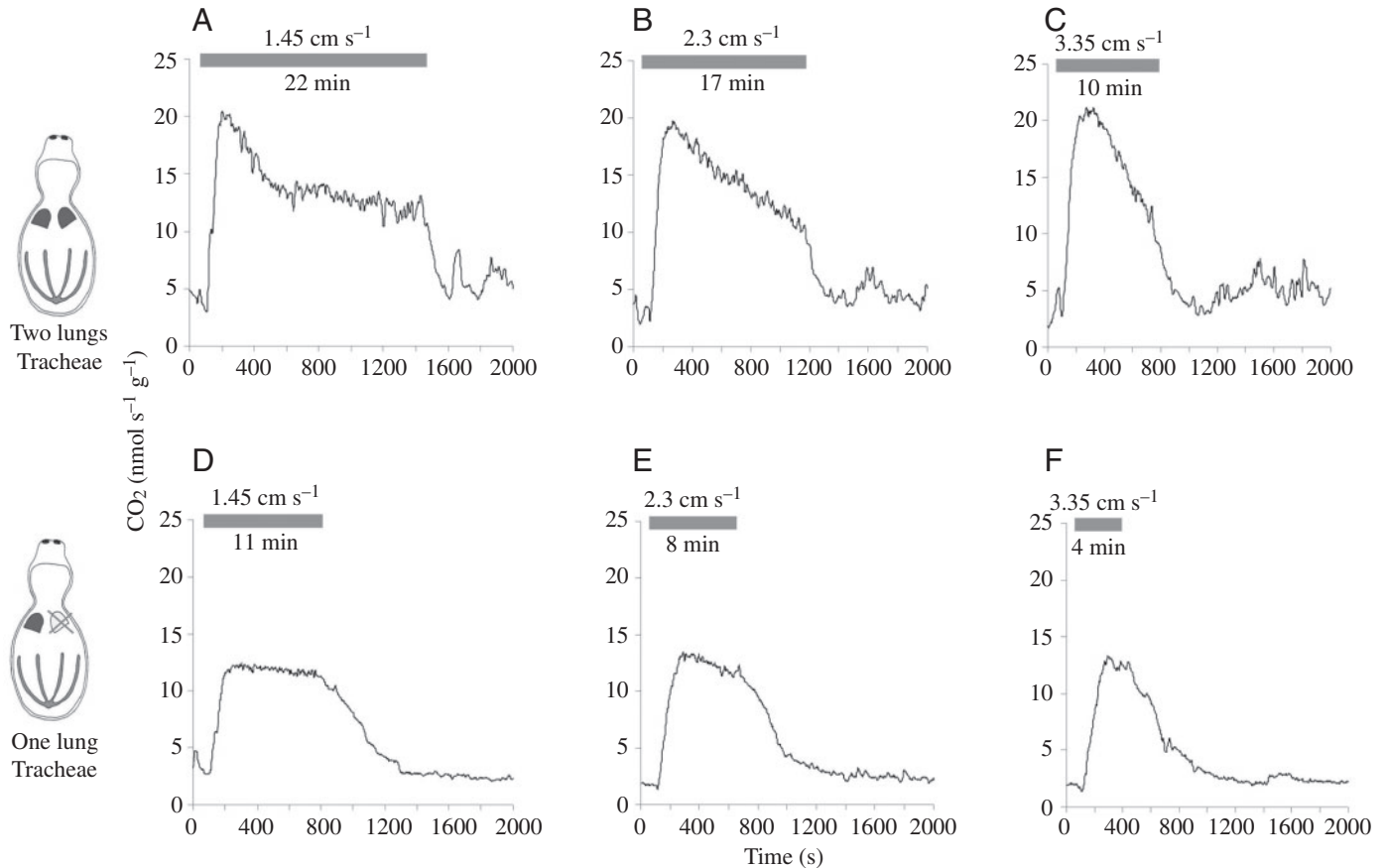


Fig. 1. Example of one *Pardosa lugubris* female running on the treadmill at the three tested speeds with intact respiratory organs (A–C) and with one sealed lung (D–F). The CO<sub>2</sub> release is marked by the black line. Duration and speed of running on the treadmill are indicated by the grey bar. The activity before and after the treadmill experiment was not evaluated.

body mass as covariance,  $P > 0.05$ ) so that both sexes were evaluated as one test group per species.

Sealing of both lung spiracles in *P. lugubris*, and of both lung spiracles and of the tracheal spiracle in *M. muscosa*, caused the death of the animal within less than 1 h. *M. muscosa* lived for at least 1 day with sealed lung spiracles when animals were in rest, but died within hours when animals were enforced to move. Sealing of one lung and the tracheae in *M. muscosa* caused the animals to show only very short and inconstant runs on the treadmill so that these experiments were not incorporated in the evaluation.

Intact animals of both species mostly started with a burst-like maximum CO<sub>2</sub> release, which then decreased to the steady state value or decreased constantly (Figs 1, 2, Table 1). Sealing of one lung decreased the maximum values by more than 30% in *P. lugubris* and eliminated the initial peaks, while in *M. muscosa* the effect was slightly smaller with one lung sealed and only remarkable at the highest speed when the tracheae were blocked (Table 1).

At the lowest speed, most individuals of intact *P. lugubris* reached a steady state  $\dot{V}_{\text{CO}_2}$  (nmol s<sup>-1</sup> g<sup>-1</sup>) (Fig. 1A, Table 1). But when one lung spiracle was sealed, CO<sub>2</sub> release values were in steady state or slightly decreased over the running time

(Fig. 1D). At the medium speed, only 70% and at the highest speed 10% of the tested intact *P. lugubris* reached a steady state CO<sub>2</sub> release while the other individuals showed a decreasing  $\dot{V}_{\text{CO}_2}$  over the running time (Fig. 1B,C,E,F). *M. muscosa* normally reached a steady state gas exchange at low and medium speeds (Fig. 2A,B, Table 1), and 30% of the animals also did so at the highest speed. When tracheae were sealed, steady state was reached by all individuals at the lowest speed (Fig. 2G), by 70% of the individuals at the medium speed and by about 20% of the individuals at the highest speed. Sealing of one lung resulted in steady state or in decreasing values over the time at the first two speeds (Fig. 2D–F, Table 1).

The steady state  $\dot{V}_{\text{CO}_2}$  increased with the three speeds tested in intact *P. lugubris* and in intact *M. muscosa* (Table 1) and in *M. muscosa* with sealed tracheae (ANCOVA,  $P < 0.01$ ). In *M. muscosa* with one sealed lung, there was also an increase in steady state  $\dot{V}_{\text{CO}_2}$  between the two lower speeds (ANCOVA,  $P < 0.01$ ). Sealing of one lung in *P. lugubris* caused a reduction in steady state  $\dot{V}_{\text{CO}_2}$  or a complete lacking of this feature (Table 1). But in *M. muscosa* only lung sealing at the medium speed and tracheal sealing at the highest speed caused a significant reduction of steady state  $\dot{V}_{\text{CO}_2}$  (Table 1).

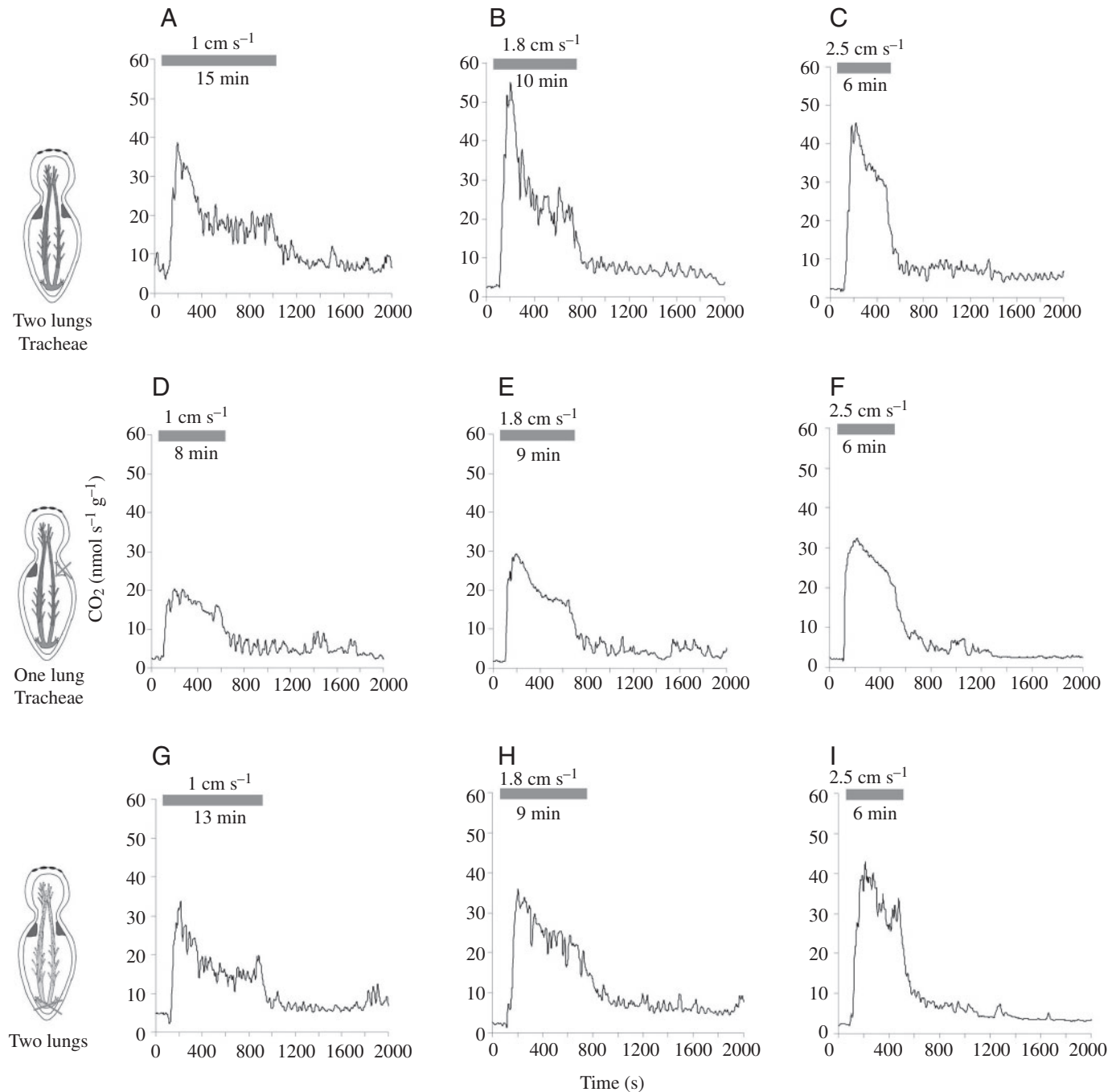


Fig. 2. Example of one *Marpissa muscosa* female running on the treadmill at the three tested speeds with intact respiratory organs (A–C), with one sealed lung (D–F) and with sealed tracheae (G–I). Duration and speed of running on the treadmill are indicated by the grey bar. The activity before and after the treadmill experiment was not evaluated.

The minimal costs of transport ( $C_{\min}$ ) were calculated from the steady state values and were determined from 2–3 speeds depending whether the individuals reached a steady state at the highest speed (Table 2).  $C_{\min}$  is higher in intact *M. muscosa* compared with *P. lugubris* (ANCOVA,  $P < 0.01$ ). Sealing of the tracheae or one lung caused a reduction of  $C_{\min}$  in *M. muscosa*, while sealing of the tracheae in *P. lugubris* had no effect on  $C_{\min}$ . As steady state CO<sub>2</sub> release did not occur at the two

higher speeds,  $C_{\min}$  was not calculable in *P. lugubris* with one sealed lung. The calculated y-intercepts (Table 2) in all test groups were 5–6 times greater than the resting rates, which are 1.8 nmol s<sup>-1</sup> g<sup>-1</sup> in *P. lugubris* and 1.4 nmol s<sup>-1</sup> g<sup>-1</sup> in *M. muscosa* (data for resting rates taken from Schmitz, 2004). The costs of transport (COT, nmol cm<sup>-1</sup> g<sup>-1</sup>) calculated from the steady state  $\dot{V}_{\text{CO}_2}$  decreased with increasing speed in both species evaluating intact animals (Fig. 3A,B). COT were

Table 1. Data for animals running at steady state CO<sub>2</sub> release

	<i>Pardosa lugubris</i>		<i>Marpissa muscosa</i>		
	Intact	One lung sealed (%)	Intact	One lung sealed (%)	Tracheae sealed (%)
<i>N</i>	24	10	30	10	16
Body mass (g)	0.030±0.009	0.029±0.013	0.029±0.008	0.0029±0.009	0.028±0.008
Steady state CO <sub>2</sub> release (nmol s <sup>-1</sup> g <sup>-1</sup> )					
LS	14.4±1.5	-19 <sup>†</sup>	16.5±3.9	-8.5	-4.0
MS	15.9±1.7*	—	21.1±4.5*	-19.5 <sup>†</sup>	-3.8
HS	18.5±0.4*	—	25.4±3.9*	—	-12.5 <sup>†</sup>
Maximum measured CO <sub>2</sub> release (nmol s <sup>-1</sup> g <sup>-1</sup> )					
LS	18.2±2.7	-32 <sup>†</sup>	29.6±9.7	-29 <sup>†</sup>	+0.6
MS	18.9±2.9	-33 <sup>†</sup>	34.5±12*	-27 <sup>†</sup>	+2.0
HS	20.4±3.2*	-35 <sup>†</sup>	37.9±11*	-29 <sup>†</sup>	-16 <sup>†</sup>

Given are the steady state values and the maximum values in CO<sub>2</sub> release for the low, the medium and the high speed (LS, MS, HS). For the intact animals, results are given as mean values (±S.D.) and the difference compared with animals with sealed respiratory organs (calculated as mean value from the individual differences) is given as percentages. \*Indicates significant difference to lower speed, <sup>†</sup>indicates significant difference to intact animals (ANCOVA, *P*<0.01).

Table 2. Slope (*C*<sub>min</sub>) and y-intercept of the regression of CO<sub>2</sub> release (nmol s<sup>-1</sup> g<sup>-1</sup>) to the speed (cm s<sup>-1</sup>)

		<i>Pardosa lugubris</i>	<i>Marpissa muscosa</i>
Intact	<i>N</i>	24	30
Body mass (g)		0.030±0.009	0.029±0.008
<i>C</i> <sub>min</sub> (nmol cm <sup>-1</sup> g <sup>-1</sup> )		2.69±1.39	8.53±4.32
y-intercept (nmol s <sup>-1</sup> g <sup>-1</sup> )		10.85±4.1	7.9±5.3
One lung sealed	<i>N</i>	10	10
Body mass (g)		0.029±0.013	0.029±0.009
<i>C</i> <sub>min</sub> (nmol cm <sup>-1</sup> g <sup>-1</sup> )		Not calculable	2.245±0.72*
y-intercept (nmol s <sup>-1</sup> g <sup>-1</sup> )		Not calculable	11.63±1.8
Tracheae sealed	<i>N</i>	16	16
Body mass (g)		0.027±0.011	0.028±0.008
<i>C</i> <sub>min</sub> (nmol cm <sup>-1</sup> g <sup>-1</sup> )		2.6±1.21	5.16±2.25*
y-intercept (nmol s <sup>-1</sup> g <sup>-1</sup> )		10.7±3.8	9.6±4.32

*C*<sub>min</sub> is given as mean value (±S.D.) of individual values and was calculated from the individual slopes of animals running in steady state CO<sub>2</sub> release at 2–3 speeds. \*Significantly different to intact animals (ANCOVA, *P*<0.01).

greater in the jumping spider and were influenced by sealing of the respiratory organs as described before for the steady state  $\dot{V}_{\text{CO}_2}$  values.

As not all animals reached steady state CO<sub>2</sub> release at all speeds, another evaluation with the data was carried out that calculates the CO<sub>2</sub> release per distance (nmol cm<sup>-1</sup> g<sup>-1</sup>) and the  $\dot{V}_{\text{CO}_2}$  (nmol s<sup>-1</sup> g<sup>-1</sup>) using the amount of CO<sub>2</sub> released during the entire period of running. The data are given in Figs 4 and 5. The CO<sub>2</sub> release per distance decreased with increasing speed in both species and in all test groups with sealed lungs or tracheae. In addition, the values were greater in *M. muscosa* in comparison with *P. lugubris* (ANCOVA, *P*<0.01). The calculated  $\dot{V}_{\text{CO}_2}$  increased with the three speeds in intact animals of both species (Figs 4B, 5B), but not in *P. lugubris* when one lung was sealed (Fig. 4D). In *M. muscosa*, the  $\dot{V}_{\text{CO}_2}$  increased between the low and the medium speed when one lung or the tracheae were sealed (Fig. 5D,F). The values for

CO<sub>2</sub> release per distance were different at all speeds in *P. lugubris* between the intact animals and the animals with the sealed lung (reduction of 33–48%) (Fig. 4A,C). In *M. muscosa* sealing of one lung reduced the entire CO<sub>2</sub> release for 14–38% at all three speeds (Fig. 5A,C), but sealing of the tracheae showed only an effect at the highest speed (Fig. 5A,B,E,F).

Evaluation of the running times and the ON- and OFF-responses revealed differences between the species and between intact and impaired animals (Table 3). The ON-response was calculated for the period from the onset of running until the animal reached half of the maximum CO<sub>2</sub> value (ON-I). In addition, a second ON-response was calculated for animals that reached steady state CO<sub>2</sub> release and was calculated from the onset of running until the animal reached half of the steady state value (ON-II). The OFF-response was calculated as the period between end of running and the time when animals reached half of the steady state CO<sub>2</sub> value. Looking at the interspecific

Table 3. Running times and the ON and OFF responses for the low, the medium and the high speed (LS, MS, HS)

	<i>Pardosa lugubris</i>		<i>Marpissa muscosa</i>		
	Intact	One lung sealed	Intact	One lung sealed	Tracheae sealed
<i>N</i>	30	16	34	16	20
Body mass (g)	0.031±0.009	0.0295±0.011	0.030±0.009	0.029±0.007	0.028±0.007
	Difference (%)		Difference (%)		
Running time (s)					
LS	793±300	-37 <sup>†</sup>	724±160	-22 <sup>†</sup>	-3.6
MS	645±271*	-44 <sup>†</sup>	565±172*	-32 <sup>†</sup>	-9.8
HS	406±133*	-44 <sup>†</sup>	304±123*	-34 <sup>†</sup>	-13.2 <sup>†</sup>
ON-I (sec)					
LS	44±9	+26 <sup>†</sup>	45±15	+28 <sup>†</sup>	+9.0
MS	52±12*	+4.5	51±17*	+4.0	+4.7
HS	55±11	+10	56±19	-14 <sup>†</sup>	-12.8 <sup>†</sup>
ON-II (sec)					
LS	28±12	+64 <sup>†</sup>	25±8	-23 <sup>†</sup>	+6.7
MS	49±14*	-	31±10*	-27 <sup>†</sup>	-10
HS	49±9	-	41±7*	-	-16 <sup>†</sup>
OFF (sec)					
LS	179±64	+201 <sup>†</sup>	120±44	+107 <sup>†</sup>	+8.0
MS	174±51	-	120±51	+69 <sup>†</sup>	-5.1
HS	170±48	-	114±46	-	+7.9

For the intact animals, results are given as mean values ( $\pm$ S.D.) and the different values of animals with sealed respiratory organs compared with intact animals (calculated as mean value from the individual differences) is given as percentages. \*Indicates significant difference to lower speed, <sup>†</sup>indicates significant difference to intact animals (ANCOVA,  $P < 0.01$ ).

comparison, only the OFF-response was different being shorter in *M. muscosa* ( $P < 0.01$ ). Sealing of one lung in *P. lugubris* caused a decrease in running time and an increase in the OFF-response (Fig. 1, Table 3) compared with the intact animals. The ON-response increased in the impaired *P. lugubris* at the lowest speed but not at the medium and high speed which resulted from the reduced maximum CO<sub>2</sub> release and the reduced entire CO<sub>2</sub> release at these two speeds. In *M. muscosa* the same effects can be found when one lung was sealed but as the percentage of difference is smaller in the jumping spider, effects seemed to be weaker than in *P. lugubris* (Fig. 2, Table 3). Sealing of the tracheae in *M. muscosa* had no effects on the OFF-response and the running time was only influenced at the highest speed. The ON-response was reduced at the highest speed because of the lower values for steady state and maximum CO<sub>2</sub> release (Table 1).

Individual comparisons of intact animals that were tested for two consecutive days showed differences in steady state  $\dot{V}_{\text{CO}_2}$ , in COT, in running times, and in the ON and OFF responses between two runs of  $\pm 10\%$  in both species.

## Discussion

### Metabolic rates during locomotion in intact animals

Both tested species are good runners and behaved well on the treadmill. Thus a constant run with constant speeds could be tested, which made a comparison of intact and restricted animals possible. The direct comparison between the species,

however, is difficult as they differ considerably in leg length and, therefore, in running speeds and running ergonomics. The maximum measured values of CO<sub>2</sub> release on the treadmill increased with speed but were smaller than the values after maximum activity, these were determined to be 26 nmol s<sup>-1</sup> g<sup>-1</sup> for *P. lugubris* and 51 nmol s<sup>-1</sup> g<sup>-1</sup> for *M. muscosa* (Schmitz, 2004). These values were evoked by shaking of an experimental chamber in which small bouncing plastic beads supported the motivation and caused animals to struggle with the beads and to escape from the experimental situation (Schmitz, 2004).

In general, spiders rely to a large extent on anaerobic metabolism during activity (Prestwich, 1983a,b). Therefore, if anaerobic metabolism in *P. lugubris* and in *M. muscosa* also occurs, the interpretation of the CO<sub>2</sub> release patterns is complicated by the production of lactic acid and the following release of CO<sub>2</sub> from solution in the haemolymph for buffering the metabolic acidosis. During running activity, animals will rapidly need more energy. Especially at the onset of activity, these energy demands might be extremely high as animals will be more excited and the running performance will be more erratic than later during activity. The initial CO<sub>2</sub> peaks during running activity might therefore be caused by an increasing CO<sub>2</sub> release because of increasing aerobic or anaerobic metabolism or a combination of both and effects will have different proportions at the different running speeds tested. The initial peaks differed largely between the tested individuals, especially in *M. muscosa* (Table 1), and without measuring the

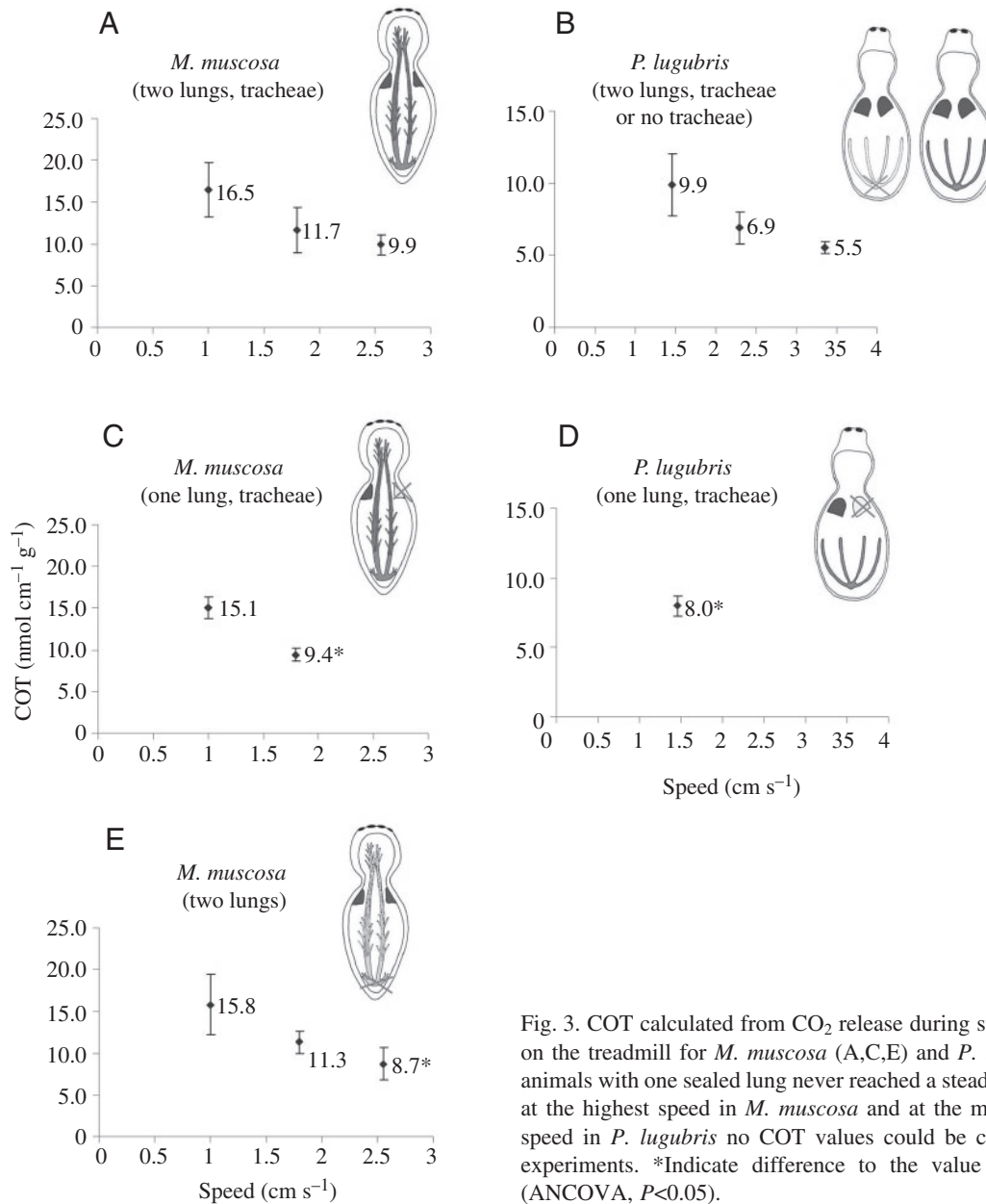


Fig. 3. COT calculated from CO<sub>2</sub> release during steady state running on the treadmill for *M. muscosa* (A,C,E) and *P. lugubris* (B,D). As animals with one sealed lung never reached a steady state CO<sub>2</sub> release at the highest speed in *M. muscosa* and at the medium and highest speed in *P. lugubris* no COT values could be calculated for these experiments. \*Indicate difference to the value of intact animals (ANCOVA,  $P < 0.05$ ).

lactic acid concentration interpretation of the peaks is difficult. In the tarantula *Eurypelma californicum*, the proportion of CO<sub>2</sub> release for buffering after intensive exercise, which causes a strong metabolic acidosis, is 34% of the total CO<sub>2</sub> release (Paul and Fincke, 1989). Values for *P. lugubris* and *M. muscosa* will certainly be lower in the speeds tested in the present paper.

Decreasing CO<sub>2</sub> release over the time (e.g. Fig. 1B,C) might result from a depletion of fluid-stored CO<sub>2</sub> during activity, the course of which is unknown in spiders, but could also be caused by a decreasing dependence on anaerobic metabolism secondary to circulatory adaptations. It can be assumed that in the tested spiders the lowest speed is supported mainly by aerobic metabolism. But the  $\dot{V}_{\text{CO}_2}$  might be increased by CO<sub>2</sub> released from the haemolymph, which is remaining from the initial anaerobic metabolism. Increasing speeds most probably

caused higher aerobic metabolism, but caused also higher anaerobic contributions to energy supply. This is supported by the results for the percentage of animals reaching steady state CO<sub>2</sub> release at the different speeds. Steady state metabolic rates themselves and increasing values with the speed indicate a great proportion of aerobic metabolism (Herreid, 1981; Shillington and Peterson, 2002). But tarantulas on a treadmill, performing strenuous activity, may reach a steady state metabolic rate even with considerable anaerobic contributions (Herreid, 1981). Because of the chosen speeds, steady state CO<sub>2</sub> release in the present study will presumably result mainly from aerobic contributions and the calculated COT and  $C_{\text{min}}$  might therefore be used for the investigation of aerobic capabilities. Conversely, the fact that not all spiders reached a steady state CO<sub>2</sub> release might be a function of the duration of

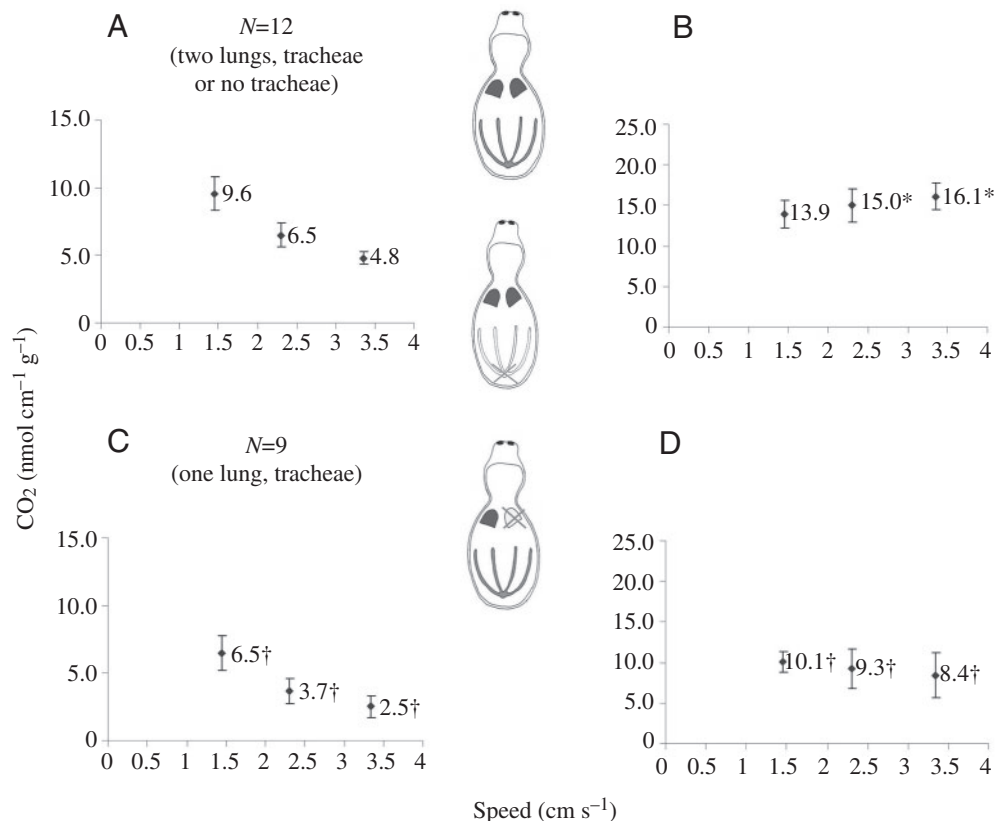


Fig. 4. CO<sub>2</sub> release per distance and the  $\dot{V}_{\text{CO}_2}$  calculated from the CO<sub>2</sub> release during running on the treadmill in *P. lugubris* with intact respiratory organs (A,B) and with one sealed lung (C,D). \*Indicate difference to lower speed and †indicate difference to the value of intact animals (ANCOVA,  $P < 0.05$ ).

running (Herreid, 1981). Thus it is possible that if runs would last long enough all animals would reach a low but steady state CO<sub>2</sub> release at the end.

Running on the treadmill under steady state  $\dot{V}_{\text{CO}_2}$  caused factorial scopes of 10 in *P. lugubris* and of 18 in *M. muscosa* (resting rates from Schmitz, 2004). For other wolf and jumping spiders and also for tarantulas, aerobic scopes during running were reported to be normally 3–10 (Miyashita, 1969; Seymour and Vinegar, 1973; Ford, 1977; Humphreys, 1977; Herreid, 1981; Prestwich, 1983b; Shillington and Peterson, 2002). All reported values higher than 6–8 are based on measurements of  $\dot{V}_{\text{CO}_2}$ , indicating considerable anaerobic proportions. But, conversely, Shillington and Peterson (2002) reported factorial scopes up to 16 during aerobic running in the tarantula *Aphonopelma anax*.

The COT were greater in *M. muscosa* compared with *P. lugubris*, which could be a function of generally improved aerobic capabilities in the jumping spider. But as shown above, the anaerobic contributions to the steady state CO<sub>2</sub> release are not known in *P. lugubris* and in *M. muscosa* and have to be tested in these species before final conclusions can be made. Furthermore, the COT declined with speed in both species. This is true in most other pedestrian invertebrates and vertebrates, as well, and is a function of the y-intercept value. For animals with large y-intercepts of more than two times the

resting rate, e.g. the cockroach species *Periplaneta americana* and the tarantula *Aphonopelma anax* (Herreid, 1981; Herreid and Full, 1984; Full et al., 1990; Shillington and Peterson, 2002), the decline of cost of transport with speed is striking. Large y-intercepts might be explained by a considerable excitement and resulting experimental stress, by increasing anaerobic contributions with increasing speed, by postural costs or a non-linearity between metabolic rate and speed (Herreid, 1981; Berrigan and Lighton, 1994).

Mass-specific minimal cost of terrestrial locomotion ( $C_{\text{min}}$ ) is a useful value for comparing the metabolic costs of pedestrian locomotion of animals with different body mass and with different numbers and length of legs that run at different speeds and have different standard metabolic rates (Taylor and Heglund, 1982; Taylor et al., 1982; Full, 1987; Full et al., 1990; Gatten et al., 1992). In addition,  $C_{\text{min}}$  can also be used as a unit of measurement for the comparison of intact and restricted animals with sealed respiratory organs.  $C_{\text{min}}$  can be predicted for vertebrates and arthropods according to the equation:  $C_{\text{min}} = 10.8 M^{-0.31}$ , where  $C_{\text{min}}$  is in J kg<sup>-1</sup> m<sup>-1</sup> and M is in kg (Full et al., 1990). The confidence interval for this equation is quite large so that costs of transport may vary 6-fold at a given body size and smaller animals with short legs should have higher metabolic costs per unit mass than larger animals. This is because of the necessary greater number of steps to travel a given

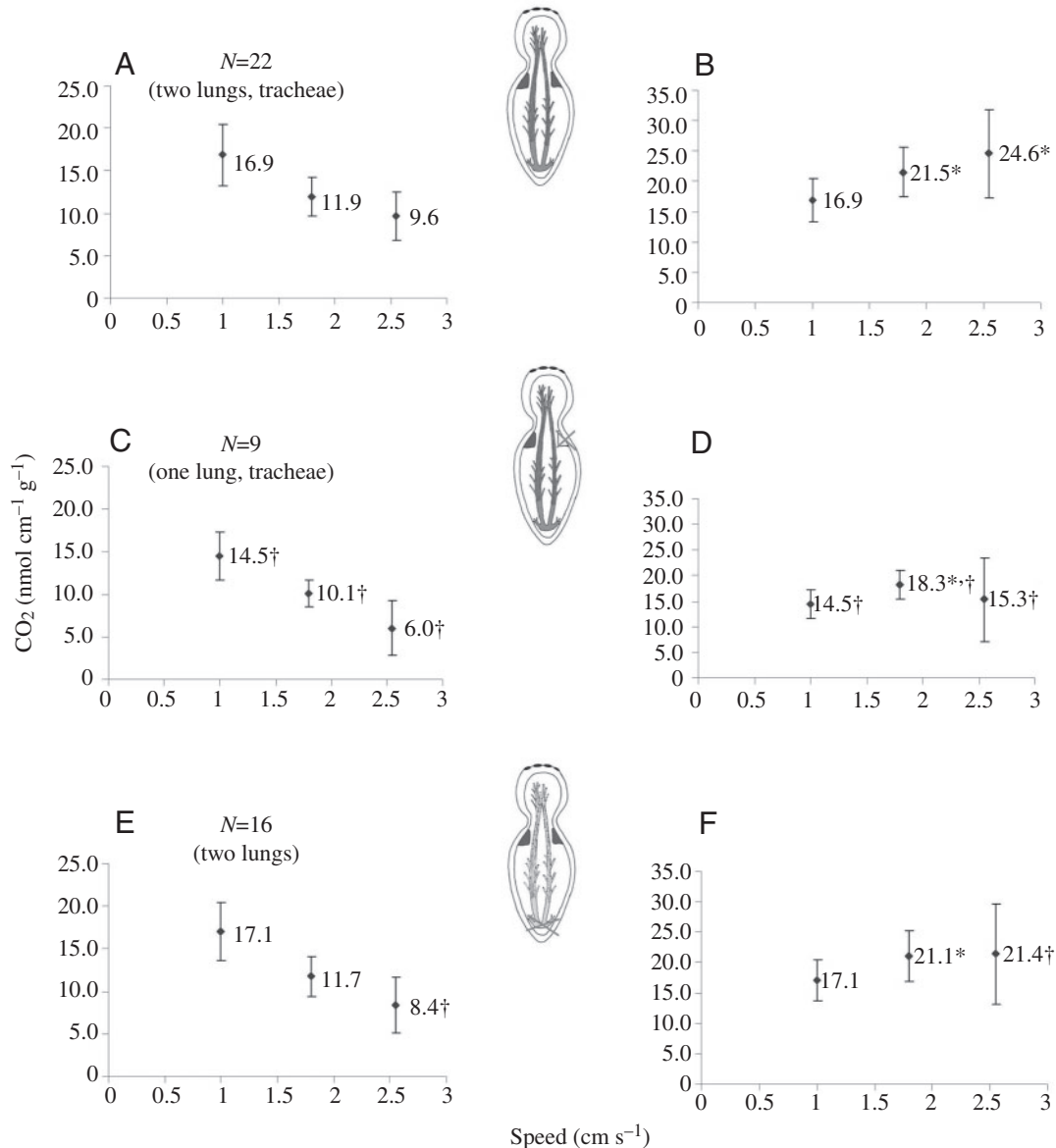


Fig. 5. CO<sub>2</sub> release per distance and the  $\dot{V}_{CO_2}$  calculated from the CO<sub>2</sub> release during running on the treadmill in *M. muscosa* with intact respiratory organs (A,B), with one sealed lung (C,D) and with sealed tracheae (E,F). \*Indicate difference to lower speed and †indicate difference to the value of intact animals (ANCOVA,  $P < 0.05$ ).

distance (Full et al., 1990). Mass specific prediction for *M. muscosa* and *P. lugubris* (mean body mass 30 mg) would result in a  $C_{min}$  of 4.05 nmol CO<sub>2</sub> cm<sup>-1</sup> g<sup>-1</sup>. The determined values for the intact animals thus fit well with this prediction within the confidence interval, indicating that body mass, leg length and resulting metabolic costs coincide with the mean value for running animals.  $C_{min}$  is smaller in *P. lugubris* compared with *M. muscosa* which will be a function of the longer legs in the wolf spider and additionally because of a possible higher anaerobic contribution. Moreover, the  $C_{min}$  in spiders can be extremely low when spiders run at very high speeds and the anaerobic contributions to metabolism are high. This was found in tarantulas in which the  $C_{min}$  was only about 15% of the predicted value (Herreid, 1981). However, testing the  $C_{min}$  at

aerobic speeds it fits well in the predicted values, as evaluated for *Aphonopelma anax* (Shillington and Peterson, 2002).

The comparison of the COT with the calculated CO<sub>2</sub> release per distance and of the steady state  $\dot{V}_{CO_2}$  with the CO<sub>2</sub> release per time during the entire activity (Table 1, Figs 3–5) reveals very similar values. This is probably a function of the equalization of the peak CO<sub>2</sub> values by the delay in CO<sub>2</sub> increase. Thus, calculation of the entire CO<sub>2</sub> release might be useful for the evaluation of metabolism during running activity and might be used for the determination of anaerobic contributions in the case that lactic acid concentrations are measured in forthcoming studies.

The running times decreased with increasing speeds, indicating increasing anaerobic contributions with speed.

Running times were about 10–15 min at the low and medium speed. Even if there is lactic acid production it is probably not overwhelming in which case clearly shorter activity times would be expected. Running times were longer in *P. lugubris*, but *M. muscosa* was often more active after the treadmill experiments. Thus interpretation of this point is difficult as animals stopped running voluntarily and thus in many cases it could hardly be decided whether animals stopped running because of exhaustion or because they did not like to continue running.

Fast ON-responses of CO<sub>2</sub> release and peak CO<sub>2</sub> values during activity reveal an ongoing circulation during activity, which makes a fast CO<sub>2</sub> release possible. Assuming a dominating aerobic metabolism during steady state CO<sub>2</sub> release, a short OFF response is a good indicator for a low O<sub>2</sub> debt. But considerable anaerobic contribution would result in a depletion of fluid-stored CO<sub>2</sub> and thus in a low CO<sub>2</sub> release from the animal although O<sub>2</sub> consumption is high because of a large O<sub>2</sub> debt.

#### *The influence of the elimination of respiratory organs*

The elimination of the tracheae in *P. lugubris* has no influence on the CO<sub>2</sub> release or all other measured parameters, indicating that tracheae have no influence on the metabolic rate during running. By contrast, the elimination of one lung caused significant differences in running time, OFF-response, the COT and the overall CO<sub>2</sub> release. Additionally, the CO<sub>2</sub> release did not increase with speed in restricted animals. Assuming predominate aerobic metabolism at the lowest speed, these results indicate that the single lung already worked at its limit at the lowest speed and anaerobic contributions became more important at increasing speeds. Lacking initial peaks in the first phase of running might be due to the lacking respiratory surface by blocking one lung. Conversely, as the peak CO<sub>2</sub> values are most probably caused by a combination of increasing anaerobic and aerobic metabolism in the restricted animals, even if more CO<sub>2</sub> is driven out from the haemolymph, the reduced CO<sub>2</sub> production by the reduced lung capacity will reduce the overall CO<sub>2</sub> release value.

The morphological oxygen diffusing capacity (diffusive conductance) of both lungs of *P. lugubris* is about 10 nmol s<sup>-1</sup> g<sup>-1</sup> kPa<sup>-1</sup> (Schmitz and Perry, 2002). Thus, using an RQ of 0.7, the calculated steady state  $\dot{V}_{\text{CO}_2}$  at the highest speed is 26 nmol O<sub>2</sub> s<sup>-1</sup> g<sup>-1</sup> and a  $\Delta P_{\text{O}_2}$  of 2.6 kPa over the lungs is necessary. Sealing of one lung resulted in maximum steady state values of 16.6 nmol O<sub>2</sub> s<sup>-1</sup> g<sup>-1</sup> which needs a  $\Delta P_{\text{O}_2}$  of 1.7 kPa at the single lung. Heart rate thus should not have been elevated during running and the O<sub>2</sub> deficit can be paid back after a shortened running and a prolonged recovery period.

In *M. muscosa*, sealing of one lung resulted in reduced  $\dot{V}_{\text{CO}_2}$  and running times and caused prolonged OFF-responses. For the lowest, presumably mainly aerobic, speed this would indicate increasing anaerobic proportions. Initial, but reduced, CO<sub>2</sub> peaks often occurred, indicating that the gas exchange capacity of the tracheae are responsible for these results

compared with *P. lugubris*. Lungs in the jumping spider, *Salticus scenicus*, have an oxygen diffusing capacity of about 9 nmol s<sup>-1</sup> g<sup>-1</sup> kPa<sup>-1</sup> (Schmitz and Perry, 2001). Assuming similar values in *M. muscosa*, the highest steady state metabolic rate in intact animals would need a  $\Delta P_{\text{O}_2}$  of about 4 kPa at the lungs. Sealing of one lung would result in a necessary  $\Delta P_{\text{O}_2}$  of 8.7 kPa. But tracheae deliver an additional oxygen diffusing capacity of about 4 nmol s<sup>-1</sup> g<sup>-1</sup> kPa<sup>-1</sup> via the tracheal walls. Thus the entire oxygen diffusing capacity of the intact animals is 13 nmol s<sup>-1</sup> g<sup>-1</sup> kPa<sup>-1</sup> and about 8.5 nmol s<sup>-1</sup> g<sup>-1</sup> kPa<sup>-1</sup> in animals with one sealed lung. This would result in a  $\Delta P_{\text{O}_2}$  of 2.8 kPa at the respiratory organs in intact animals and in animals with one sealed lung at the respective maximum steady state metabolic rates. The metabolic rates with one sealed lung therefore can be reached by compensation via the tracheae or alternatively by an increased heart rate to increase the  $\Delta P_{\text{O}_2}$  at the lungs.

Sealing of the tracheae in *M. muscosa* did not influence the  $\dot{V}_{\text{CO}_2}$  and the running time at the low and the medium speed. Thus tracheae seem to be not involved in gas exchange at low and mean activity and the greater  $\dot{V}_{\text{CO}_2}$  of *M. muscosa* in comparison with *P. lugubris* will be independent of the tracheae. At the highest speed, however, sealed tracheae reduced the  $\dot{V}_{\text{CO}_2}$ , but values were still greater than in *P. lugubris*. Taking into account that the lungs of both species have similar diffusing capacities, these results indicate that tracheae only partly support the higher  $\dot{V}_{\text{CO}_2}$  in *M. muscosa* compared with *P. lugubris*. Moreover, eliminated respiratory organs reduced the C<sub>min</sub> in *M. muscosa* which indicates higher anaerobic contributions. The effect was greater with one sealed lung (75% reduction) than with the sealed tracheal system (40% reduction). Because in one-lunged *P. lugubris*, C<sub>min</sub> cannot be calculated at all, this is another hint that tracheae in *M. muscosa* can be used for compensation when lung capacity is not sufficient. But results of C<sub>min</sub> should be interpreted with care, as in many individuals with sealed spiracles it was calculated only from two speeds, which might have produced diverging results.

Comparative testing of the maximum activity in wolf and jumping spiders revealed that the corresponding jumping spider (*P. audax*, *M. muscosa*) showed higher maximum metabolic rates, shorter ON- and OFF-responses and lower anaerobic contributions than the wolf spider (*L. lenta*, *P. lugubris*) (Prestwich, 1983b; Schmitz, 2004). Together with the data shown in the present paper it can be assumed that the investigated jumping spiders have greater aerobic capabilities than the respective wolf spiders, but that tracheae alone are not responsible for these differences. Tracheae in jumping spiders seem not to change in principal the strategy of using anaerobic capabilities during fast running in spiders (Prestwich, 1983a,b), but the role of anaerobic metabolism in small spiders, as *P. lugubris* and *M. muscosa*, is still a matter of speculation and should be tested for a better understanding of the presented results. In addition, also other factors have to be considered as playing a role in aerobic capabilities. Such factors are the morphology and physiology of the circulatory system, the function of the respiratory pigment hemocyanin in the

haemolymph, and the number and density of mitochondria in the exercise muscles that all have not been investigated to date.

#### *How do tracheae in jumping spiders function during physical exercise?*

Tracheae in jumping spiders end in the nervous system, the gut epithelium or in the haemolymph (Schmitz and Perry, 2000). But 70–80% of the tracheal surfaces are in contact with the haemolymph (Schmitz and Perry, 2001), in which haemocyanin is available as respiratory protein (Markl et al., 1986; Schmitz and Paul, 2003). The gas exchange from the tracheal system to the tissue may therefore function according to two principles: (1) the principle of lateral diffusion in which gas exchange takes place *via* the walls of the entire tracheal system, (2) the principle of terminal diffusion in which gas exchange takes place at the distal endings in the haemolymph or in the tissue itself. Jumping spiders most probably use a mixture of the two principles. Tracheae seem to be not necessary in maintaining metabolic rates of the exercising muscles during low and medium activity. But they support the local oxygen supply to the gut, which should be of no importance during exercise, and to the nervous system, which is of great importance for the support of the eyes. Jumping spiders have excellent visual capabilities which are essential during exercise, and could, in addition, be one reason for the higher metabolic rates in these spiders. The importance of the tracheae for the eyes is also demonstrated by the refusal to run constantly on the treadmill when one lung and the tracheae were sealed. At low and medium activity and when tracheae are sealed, lungs can provide enough oxygen, also for the eyes, *via* the haemolymph, probably supported by an increased engagement of the circulatory system. At high activity, however, tracheae seem to be necessary for maintaining metabolic rates and the lacking tracheal capacity can only be partly compensated by the lungs.

In conclusion, the results of the present paper are consistent with the hypothesis that tracheae partly support the enhanced aerobic capabilities of jumping spiders compared with the two-lunged wolf spiders. Looking at the anatomy, tracheae might be mainly responsible for the local oxygen supply, especially of the nervous system. Physiological results, however, revealed a joint responsibility of the tracheae for the metabolic rates during strong physical exercise, which could be a combination of the needs for muscular activity and for the processing of visual input. Tracheae can fulfil both demands because of the possibilities of gas exchange *via* the tracheal system, which may function in local oxygen delivery by penetrating organs and by global oxygen delivery by gas exchange *via* the tracheal walls incorporating the haemolymph in gas transport. Further investigations have to reveal the exact role of anaerobic metabolism and the role of the circulatory system in maintaining the metabolism during exercise in small araneomorph spiders.

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