ORIGINAL PAPER

Leg loss decreases endurance and increases oxygen consumption during locomotion in harvestmen

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Abstract

Animal movements are highly constrained by morphology and energetics. In addition, predictable bodily damage can constrain locomotion even further. For example, for animals moving on land, losing legs may impose additional costs. We tested if losing legs affects the distance travelled over time (endurance) and the metabolic costs of locomotion (oxygen consumption) in *Nelima paessleri* harvestmen. These arachnids voluntary releases legs (i.e., autotomy) in response to predation attempts. We used flow-through respirometry as animals moved on a treadmill inside a sealed chamber. We found that endurance decreased gradually with an increasing number of legs lost. Interestingly, oxygen consumption increased only for harvestmen that lost three legs, but not for individuals that lost only a single leg. These results have different ecological and evolutionary implications. Reduced endurance may impair an animal's ability to continue moving away from potential predators, while increased oxygen consumption makes movement costlier. Our findings suggest that individuals have a threshold number of legs that can be lost before experiencing measurable energetic consequences. Overall, our findings illustrate how animals respond to morphological modifications (i.e., damage) that affect the physiology of locomotion.

Keywords Autotomy · Locomotion energetics · Opiliones · Respirometry

Introduction

Morphological damage can affect movement (Maginnis 2006; Fleming et al. 2007), which is an already highly constrained process in animals (Herreid et al. 1981a; Full 1991). To move, an animal needs resources (e.g., carbohydrates and oxygen) to produce the energy required to displace its body. Animals also need structures capable of generating the physical forces necessary for movement (thrust, lift, energy exchange) (Jindrich and Full 1999; Vogel 2013). This requires maintaining complete and healthy appendages such

as legs, tails, or wings. Consequently, any damage or loss to those appendages could impact movement negatively. Movement is also performed in environments that likely impose additional challenges such as competitors or predators (Somjee et al. 2018; Wilshin et al. 2018). Thus, movement is a complex, multi-dimensional process that reflects the outcome of numerous selective pressures acting on morphology, physiology, and behavior.

Damage on appendages associated with movement may impact survival and fitness. One type of common bodily damage occurs when animals voluntarily release an appendage or a body part to escape potential predators. This phenomenon, known as autotomy, includes loss of legs, wings, or tails at specific breakage planes (reviewed in Emberts et al. 2019). While this self-inflicted bodily damage is expected to increase immediate survival, it may negatively affect other aspects of organismal function such as energetics of locomotion (Emberts et al. 2019). For example, loss of body parts may impact endurance, defined as the ability to sustain continuous and uninterrupted locomotion over time and distance (Weinstein and Full 1999; Allen and Levinton 2007; Fleming and Bateman 2007; Fleming et al. 2009). After tail loss, geckos

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moved for a shorter distance (Fleming et al. 2009), and sustained movement for a shorter time (Lu et al. 2010); crickets moved a shorter distance and stopped more often after leg loss (Fleming and Bateman 2007).

Autotomy may also affect the energetic input required to produce movement, which frequently measured as O_2 consumption or CO_2 production (Bartholomew et al. 1981; Full 1991; Biro and Stamps 2010; Gast et al. 2019). When corrected for time, body weight, and distance travelled, these measures describe the metabolic cost of locomotion. The amount of oxygen consumed while moving scales linearly with speed and decreases with body size (Herreid et al. 1981a, b; Herreid and Full 1986; Full 1991; Schmitz 2005). Additionally, the metabolic costs of locomotion are associated with kinematic performance and selection on morphological traits (Allen and Levinton 2007; Somjee et al. 2018; O'Brien et al. 2019). These measures have been used to examine post-autotomy changes in the energetic of locomotion in animals. For instance, after autotomy, O_2 consumption increased in hermit crabs (Herreid and Full 1986) and blue crabs (Mcgaw 2006). Similarly, CO_2 emissions increased in crickets (Fleming and Bateman 2007) and geckos (Fleming et al. 2009), and the resting metabolic rate (RMR) increased in lizards (Naya et al. 2007) and *Heliconia* bugs (Somjee et al. 2018) after autotomy.

To date, experimental studies of the effects of variable levels of autotomy-caused bodily damage to endurance and metabolic costs have been scarce. These relationships are critical to understanding the physiological consequences of autotomy as well as the ecological and evolutionary implications of autotomy and bodily damage (Emberts et al. 2019). As animals experience greater levels of autotomy, greater negative consequences are expected. Additionally, for taxa that experience high rates of autotomy, compensatory mechanisms may have evolved to alleviate the consequences of that bodily damage. As a result, losing one versus multiple legs may have different life-history implications.

We explored the influence of variable levels of leg loss on the energetics of locomotion in harvestmen (Arachnida: Opiliones). Harvestmen are ideal organisms for exploring the causes and consequences of autotomy. Many species autotomize legs as a defensive strategy (Escalante et al. 2013, 2020; Domínguez et al. 2016) and individuals do not regrow legs, even if loss happens before reaching adulthood (Shultz and Pinto-da-Rocha 2007). Harvestmen use aerobic metabolism while moving (Phillipson 1963; Anderson 1993; Schmitz 2005; Lardies et al. 2008). Spiracles in each walking leg (Höfer et al. 2000) intake oxygen that is then distributed to cells and tissues via continuous tracheal gas exchange (Lighton 2002; Shultz and Pinto-da-Rocha 2007). Importantly, contrary to other arachnids that make extensive use of hydraulic pressure (Weihmann et al. 2012; Hao et al. 2019), harvestmen legs move by muscle contractions

(Shultz 2000; Sensenig and Shultz 2003) that require high oxygen input.

Here, we tested the hypothesis that leg loss affects the energetics of locomotion in the harvestmen *Nelima paessleri* Roewer 1910 (Opiliones: Sclerosomatidae). We experimentally induced varying levels of autotomy (number of legs lost) and assessed the effects of this manipulation on endurance and oxygen consumption during locomotion on a treadmill. We predicted that autotomy would lead to (1) decreased endurance and (2) increased oxygen consumption compared to intact individuals. Finally, we expected (3) higher costs in both endurance and oxygen consumption with an increasing number of legs lost. Overall, this study provides novel insights into the potential trade-off between adaptive damage via leg loss and crucial aspects of locomotion (energetic input and performance).

Methods

Animals

Harvestmen of *N. paessleri* were collected at the Lower Fire Trail (37° 52′22″ N 122° 14′20″ W, 210 m in elevation) in Berkeley, Alameda Co., California, USA, on the unceded lands of the Chochenyo and Ohlone people. From March to September 2018, animals were found roosting in the crevices of large (1 m diameter) concrete drainage pipes that run underneath the trail. For each adult, we recorded its sex and number of legs at time of capture. We assessed sex via morphological proxies (body size and shape) based on ten previously dissected individuals where internal genitalia was identified. Only intact (eight-legged) sexually mature adult harvestmen ($N=60$) were transported to the lab for the experiment. In the lab, animals were individually housed in a terrarium ($20 \times 10 \times 15$ cm) with soil and leaf litter, and were fed ad libitum quantities of wet cat food and fruit, and misted water was provided every 2 days.

Experimental setup

Animals were tested while moving on a treadmill located inside a 550 cm^3 (14 cm long × 7.5 cm wide × 5 cm in radius) sealed Plexiglas respirometer half-cylinder chamber, as described in Kram et al. (1997) (Fig. 1, Online Resource 1). This allowed us to visually record endurance (see below) and quantify oxygen consumption. We used a S3-A II Applied Electrochemistry Oxygen Analyzer to measure percent-oxygen concentration flowing in and out of the chamber, with an inflow rate calibrated to 20.94% O_2, and a flow rate of 70 ml/ min. Two cells measured oxygen concentration: one cell acted as a reference channel, where gas flowed through, but did not enter the treadmill chamber, while the second cell

Fig. 1 Open-flow respirometer used for harvestmen (*Nelima paessleri*) experiments. See "Methods" for further description of the procedures used to measure endurance and oxygen consumption
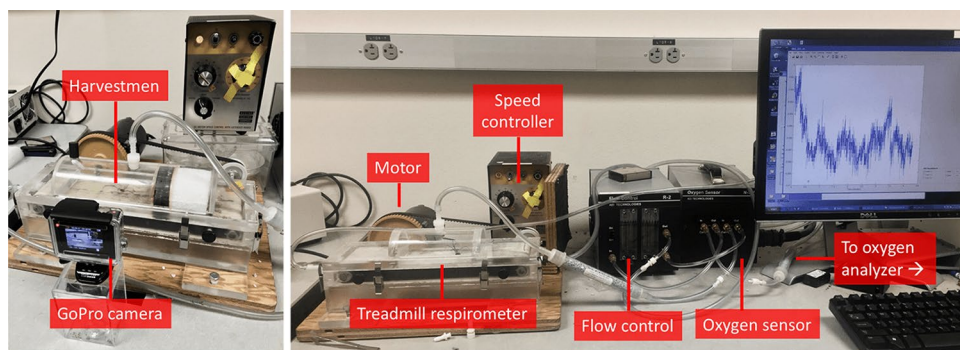
measured oxygen concentration flowing out of the metabolic chamber containing the treadmill and focal animal. Oxygen concentration was captured by a custom data acquisition script on MATLAB (The Mathworks, Natick, MA, USA). By subtracting the concentration of the air flowing in minus the concentration on the air flowing out, the script reported oxygen consumption.

Pilot observations confirmed that *N. paessleri* harvestmen were able to move unencumbered on the treadmill. While doing so, they used either the running or walking gaits described for another species of harvestmen (Escalante et al. 2019). Briefly, when harvestmen move they alternate the use of two leg tripods (legs 1L-3R-4L and 1R-3L-4R), by having with one tripod on the ground and the other in the air at a given time (Sensenig and Shultz 2006; Escalante et al. 2019).

Trials began at least 20 min after introducing the animal to the chamber as opening it resulted in air mixing and tubbing which interferes with accurate measurements. This also allowed animals to acclimate after stress from handling and introduction to their new surroundings. The delay also ensured gas equilibrium, and that the animal's respiration is the only source of change in oxygen levels. Oxygen concentration in the chamber was recorded for 10 min before the treadmill was turned on to obtain a reading of the animal's resting metabolic rate (RMR). Harvestmen did 2–3 short bouts of < 10 s walking during the first minute in the chamber. After that, we observed that harvestmen remained motionless during that time when the treadmill was off.

The initial velocity for the treadmill was started at 20 revolutions per minute (RPM) (~ 2.09 m/s). After 20 s, velocity was increased to 25 RPM (~ 2.6 m/s), and after an additional 20 s, increased again to 30 RPM (~ 3.1 m/s). Animals moved as long as the treadmill was active up to several minutes (range 6–16 min) (mean ± standard deviation: 696 ± 140 s). After the treadmill was turned off, the animals rested without moving inside the chamber. After we visually confirmed that respiration returned to RMR, we removed the harvestmen from the chamber and returned them to their home terrarium. RMR pre-exercise (mean ± SE = 4.03 ± 0.23 ml/h/g, $n = 120$) and post-exercise (4.01 ± 0.20 ml/h/g, $n = 120$) were

statistically indistinguishable across trials and treatments (General Linear Model: $P > 0.31$ for the predictor factors pre/post-exercise, trial, treatment, and all their interactions. Model had individual as random factor).

We video-recorded the trials using a GoPro Hero 6 camera at 1080p resolution and 120 frames per second. The camera was placed on a tripod 10 cm from the chamber (Fig. 1) on lateral view, with the treadmill motor control unit in the frame to record changes in treadmill velocity.

Experimental manipulation of leg number

We ran each adult on the treadmill when they were intact (with eight legs) for the 'before' trial. Animals were then kept in their terrarium until their second trial ('after'), conducted 2 days later. We induced autotomy as described in Escalante et al. (2020) 6 h before the second trial. To do so, we held the animal by grabbing all of its legs by the tibias. We then firmly grabbed the base of the femur with forceps, which resulted in the quick (< 1 s) release of the leg from the coxae–trochanter joint. This imitates the natural response when a harvestman is grabbed by a potential predator.

All harvestmen ($n = 60$) were randomly assigned to one of three treatments: (i) autotomy of one leg from the leg I pair (1L), (ii) autotomy of three locomotor legs, two from the leg I pair and one from the leg III pair (3L), or (iii) control (C), in which we handled individuals in the same way but without inducing autotomy. We chose these treatments based on our previous findings in a related species that showed loss of three legs negatively affected locomotor performance, while the loss of a single leg did not (Escalante et al. 2020). We did not induce autotomy of leg II as they function as sensory appendages (Willemart et al. 2009), and are not used for locomotion in intact harvestmen (see Fig. 4 in Escalante et al. 2019). Missing one leg (1L treatment) is fairly common in the field (Escalante et al. 2020, and this project), and thus, we expected harvestmen to compensate and show minimal effects on the energetics of locomotion. Because missing both legs I and one leg III (3L treatment) is uncommon in the field, we predicted significant changes following autotomy on the energetics of locomotion.

Quantifying endurance

We measured endurance as the total distance travelled (Fleming and Bateman 2007; Fleming et al. 2009; Grossi et al. 2016) by the harvestmen in the trial. To do so, we observed harvestmen for signs of fatigue (Weinstein and Full 1999), i.e., cessation of movement or failure to keep pace with the treadmill (Fig. 1). When the animal displayed fatigue, we first decreased the treadmill speed from 30 to 25 to 20 RPM, and then stopped the treadmill for 3 s and re-started it. Since endurance decreases with speed (Full 1987; Full and Tullis 1990); and given individual variation in the length of time and the speed at which these harvestmen moved on the treadmill, we used the difference in the total distance travelled by each harvestmen (Grossi et al. 2016) as our measure of endurance. We multiplied the total time during which the animal moved on the treadmill at each of the three speeds (20, 25, and 30 RPM) by the actual speed (see above), resulting in the total distance travelled in meters. We then calculated the individual-specific difference in the distance travelled (Δ distance travelled) by subtracting the value in the after trial from the value in the before trial, and used it as the main measure of endurance.

As secondary measures of endurance, we recorded both the number of times the treadmill was stopped during each trial and the frequency of "recovery" events. When harvestmen were not able to keep up with the pace of the treadmill, their hind legs got stuck in the crevice between the treadmill and the back wall of the chamber. We noted a recovery event when the animal pulled away from that crevice and continued moving. Recovery events were confirmed in the video for each trial.

Quantifying metabolic costs of locomotion

We examined two measures of the energetic cost of locomotion. The first, resting metabolic rate (RMR), is a weight-corrected parameter that represents an average measure of oxygen consumption, while an animal is still. This allowed us to calculate resting metabolic rate (RMR) before an animal began to move, using the formula: VO_2 (oxygen consumption) = [(flow rate (ml/min)*60 min/h) * (Δ O_2%/100)]/ body mass (g), where VO_2 is measured in units of ml/h/g.

The second measure examined was the individual-specific difference in the metabolic cost of transportation before and after treadmill trials, hereafter 'Δ VO_2'. To estimate this parameter, we first divided the average oxygen consumption during exercise (Fig. 2) by the total distance travelled. This measure reports oxygen consumed in units of ml/h/g/m. This metric controls for individual variation in time spent moving and treadmill speed, since VO_2 correlates with the speed at which animals move (Herreid et al. 1981a, b; Herreid and Full 1986; Schmitz 2005). Next, we subtracted Δ VO_2 for the before trials from the values for the after trials.

For each trial, we noted several stages (Fig. 2): when the RMR was achieved after closing the chamber, when we started the treadmill, when the harvestmen was moving ('exercise'), when we turned the treadmill off, when the reading returned to the initial RMR, as well as the time it took to recover RMR (Fig. 2). As secondary measures of the metabolic costs of locomotion, we used (1) the maximum
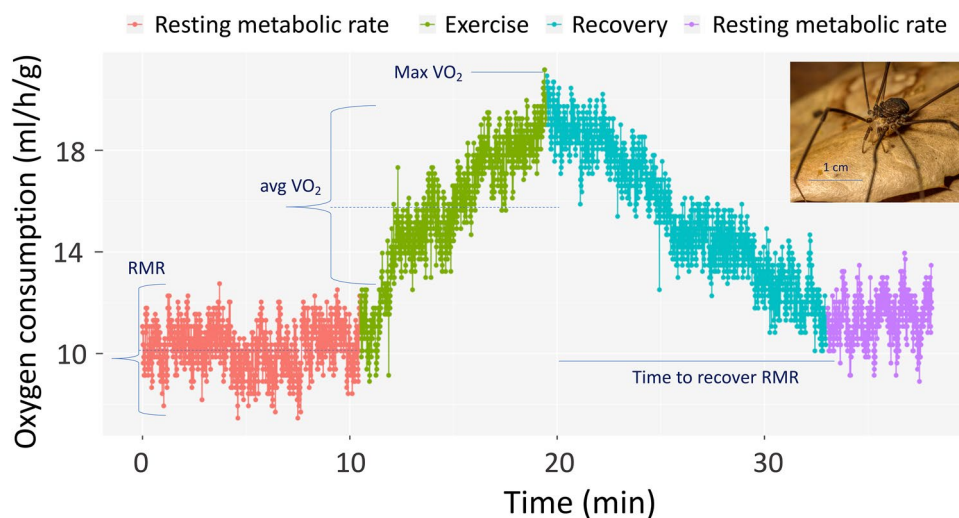

Fig. 2 Sample recording of oxygen consumption (VO_2) over time for a harvestmen (*Nelima paessleri*) while moving on a treadmill inside a sealed open-flow respirometry chamber. Sample is divided by different stages (pre-exercise, treadmill on, time to recover RMR, and return to RMR). Brackets denote the range of values used to calculate the average (dashed lines) for resting metabolic rates (RMR) and the distance-corrected average VO_2. RMR levels pre- and post- exercise were statistically indistinguishable (see "Methods" for further details). Inset: adult *N. paessleri*, picture by C. Irian

oxygen consumption while moving on the treadmill and (2) the time (in seconds) it took to recover initial RMR after the treadmill was turned off and the animal stopped moving (Fig. 2). For the latter, we subtracted the time at which RMR was achieved after exercise from the time of maximum VO_2.

Morphological measurements

To calculate oxygen consumption, we weighed each harvestmen after each trial to the nearest 0.001 mg on a digital scale. Body weights ranged from 50 to 90 mg. For the before trial, all animals weighed had eight legs and for the after trial, animals had either eight, seven, or five legs, according to the treatment. To explore the effect of morphology on the energetics of locomotion, we measured the length of leg IV to the nearest 0.05 mm with digital calipers. After the experiment, we preserved each individual in 85% ethanol. Next, we measured their dorsal body area (excluding legs) to the nearest 0.05 mm using a camera attached to a dissection scope (Leica M205 FA). All individuals were deposited as voucher specimens at the Essig Museum of Entomology, University of California, Berkeley.

Data analyses

We first tested if the frequency of leg loss in field-surveyed individuals differed among the four pairs of legs using a Chi-square. Next, we tested for normality of the response variables (endurance and metabolic costs) using Shapiro tests applied to the residuals of each response variable. To explore statistical interdependency, we conducted Pearson correlation analyses between all morphological, endurance, and metabolic cost variables. Identifying significant correlations allowed us to select only the most biologically and statistically informative variables for further analysis. The secondary measures of endurance (number of times the treadmill was stopped, and recovery events), and the metabolic costs of locomotion (max VO_2, time to return to RMR) (Online Resource 2) were not included in statistical analyses as these measures correlated with the main variables measured (Δ distance travelled and VO_2) (Online Resource 3). The Δ distance travelled and ΔVO_2 were negatively correlated ($r = -0.32$, $P = 0.01$, Online Resource 3).

Leg length was not correlated with body size, body weight, or any of the endurance or metabolic costs of locomotion variables examined (Online Resource 3). We did not include leg length in the models reported below as preliminary models showed that leg length did not differ between treatments (Table 1). Body area was correlated with body weight ($r = 0.73$, $P < 0.0001$), with two measures of endurance, and with all measures of the metabolic costs of locomotion except time to recover RMR (Online Resource 3). We did not include body area in the models as we consider

that the effects of body area were incorporated in the metabolic costs of locomotion metrics, as ΔVO_2 was calculated using body weight.

To test the hypothesis that leg loss affects the energetics of locomotion we ran a General Linear Model (GLM) to compare the Δ distance travelled across treatments (C, 1L, or 3L); and we ran a second GLM to compare ΔVO_2 across treatments. To confirm that there were no biases among treatments with regard to body size, we compared body area and leg length across treatments with Kruskal–Wallis tests. Pre- and post-autotomy values for RMR were compared using a linear mixed effect (LME) model, with trial (pre- or post-autotomy) and the interaction between trials as fixed effects and animal identity as a random effect. The complete dataset is available on Dryad (https://doi.org/10.5061/dryad.76hdr7ssv). Statistical analyses were run in R (R Core Team 2019).

Results

Autotomy in the field

We identified 1030 free-living adult harvestmen, of which 28.3% were missing legs. Of those individuals, 268 (26%) were missing one leg, while only 24 (2.3%) were missing two legs, and no animal was found missing three legs. Across all animals captured, missing limbs were detected for all pairs of legs. We found that 44% of missing limbs were from pair II, which was significantly more often than expected by chance (Chi-square: $X^2 = 11.85$, df $= 3$, $P = 0.01$, $n = 54$). Legs from pair I were missing 12% of the time, whereas legs pair III and IV were missing 21% and 23% of the times, respectively.

Effects of autotomy on endurance

The Δ distance travelled was higher with increasing number of legs lost (GLM: $P < 0.001$, Table 1). Animals that lost one leg (1L) had a Δ distance travelled of 5.3 ± 0.8 m, which represents a 22 ± 3% decrease in the distance travelled after leg loss (Fig. 3). Animals that lost three legs (3L) displayed a mean Δ distance travelled of 10.2 ± 0.7 m, which represents a 44 ± 2% decrease in the distance travelled. In contrast, control animals decreased their distance travelled by only 1.9 ± 1.2 m, or 16 ± 5% (Fig. 3, Table 1). Post hoc tests revealed that Δ distance travelled was higher for 1L than for control harvestmen, and that Δ distance travelled was higher for 3L than for the other two treatments (Fig. 3). In the after trials, the number of times the treadmill was stopped increased for both 1L and 3L, but not for control individuals (Online Resource 2). Additionally, the number

Table 1 Summary of statistical analyses of relationships among morphological, endurance, and energetic variables used to assess the effects of leg loss on the energetics of locomotion in *Nelima paessleri* harvestmen

Variable	Parameter	Control		1 Leg missing (1L)		3 Legs missing (3L)		Normal distribution? (Shapiro test)	Between treatment effects			
		Before trial	After trial	Before trial	After trial	Before trial	After trial		Test statistics	P	Post hoc tests	Patterns
Body area (mm2)	Mean±SD	20.51±3.24		20.29±3.24		18.56±3.44		0.007*	Kruskal–Wallis: $X^2=2.88$, df=2, $P=0.24$			
	Min – max	15.23 to 26.49		12.11 to 26.32		10.62 to 24.61						
Leg 4 length (mm)	Mean±SD	53.84±3.64		54.37±3.08		53.09±4.31		0.02*	Kruskal–Wallis: $X^2=0.68$, df=2, $P=0.71$			
	Min – max	47.8 to 59.63		48.52 to 59.37		45 to 59.38						
Endurance: distance travelled (Δ m)	Mean±SD	−195.5±567.6		−530.8±354.4		−1019.±333.6		0.21	GLM. $F_{2/57}=18.42$, $P<0.001*$		$P=0.04$ for C-1L, $P<0.001$ for C-3L, $P=0.002$ for 1L-3L	Greater changes for C>1L>3L
	Min—max	−1634 to 1332		−1293 to 60		−1806 to −524						
Average resting metabolic rate (RMR) (ml/h/g)	Mean±SD	3.32±1.79	3.72±2.07	3.68±2.37	3.47±2.16	3.74±1.91	4.14±2.98	<0.0001*	LME. Trial: $F_{1/57}=0.27$, $P=0.61$. Treatment*Trial: $F_{2/57}=0.29$, $P=0.75$			
	Min – max	0.78 to 9.37	1.44 to 8.54	0.69 to 8.29	0.86 to 10.12	1.33 to 7.76	0.62 to 12.07					
Δ Metabolic cost of transport (Δ avg VO2) (ml/h/g/m)	Mean±SD	0.79±1.65		1.40±2.36		3.28±3.46		0.0001 *	GLM: $F_{2/57}=4.95$, $P=0.01*$		$P=0.74$ for C-1L, $P=0.01$ for C-3L, $P=0.04$ for 1L-3L	3L treatment with higher change

Table 1 (continued)

Variable	Parameter	Control		1 Leg missing (1L)		3 Legs missing (3L)		Normal distribution? (Shapiro test)	Between treatment effects			
		Before trial	After trial	Before trial	After trial	Before trial	After trial		Test statistics	P	Post hoc tests	Patterns
	Min – max	– 2.25 to 4.17		– 2.85 to 8.30		– 2.33 to 10.7						

Sample size for all variables and treatments is 20 individuals. 'Before trial' represents the data for the first trial, in which all animals moved with eight legs. 'After trial' was the second trial, 2 days after the before trial, in which 2/3 of the animals had experience leg loss. See "Data analyses" on Methods for further explanation of the models ran. Individual identity was included as a random factor. Significant effects are marked with an asterisk

of recovery events decreased only for 3L individuals after leg loss (Online Resource 2).

Metabolic costs of locomotion

The metabolic costs of locomotion did not change linearly with number of legs lost. Individuals missing three legs (3L) had a threefold increase in average oxygen consumption (Δ VO_2) during exercise (Fig. 4; Table 1), which was significantly higher than both control (C) and animals missing one leg (1L) (GLM: $P < 0.01$, Fig. 4, Table 1). Control and 1L harvestmen did not differ in their Δ VO_2. The baseline resting metabolic rate did not differ between treatments or trials ($P > 0.61$ for both comparisons, Table 1).

Discussion

Endurance and oxygen consumption were affected by experimentally induced leg loss in *N. paessleri* harvestmen. Interestingly, these measures of locomotion energetics showed different responses. While the ability to sustain continuous and uninterrupted movement (endurance) decreased with number of legs lost, oxygen consumption only changed for animals that had lost three legs. Together, our findings demonstrate that it is not the leg loss per se that compromise the energetics of locomotion, instead the magnitude of loss; in this case, the number of legs lost.

Effects on endurance

A higher Δ distance travelled after autotomy likely reflects the impacts of leg loss on the mechanics of movement. The affected mechanisms could include decreased stability and maneuverability while moving (Jindrich and Full 1999; Hsieh 2016; Mountcastle et al. 2016), not being able to adopt the typical alternating-tripod gait (Sensenig and Shultz 2006; Escalante et al. 2019, 2020), inability to produce enough ground-reaction forces and thrust (Jindrich and Full 1999; Reinhardt and Blickhan 2014; but see Halsey and White 2019), and/or the exchange between potential and kinetic energy (Full and Tu 1991; Weihmann 2013). After losing legs, several of these scenarios may occur, with more mechanisms coming into play with increased levels of leg loss. Consequently, this might explain the gradual decrease in endurance with increasing levels of autotomy. The additional metrics of endurance (number of times the treadmill was stopped and the number of recovery events by the harvestmen) also showed greater costs in animals that lost more legs. While previous research in reptiles and insects also found decreased endurance with the loss of legs or tails (Martin and Avery 1998; Chapple and Swain 2002; Fleming and Bateman 2007; Fleming et al. 2009), our findings are

Fig. 3 Endurance of harvestmen of *Nelima paessleri* based on their experimental leg condition while moving on a treadmill inside a sealed open-flow respirometry chamber. Delta endurance represents the difference between the trial before and the trial after leg loss for the same individuals ($n = 20$ individuals per treatment). Diagram in the bottom row depicts each treatment: intact (C), missing one locomotor leg (1L), missing three locomotor legs (3L). Leg length in the diagrams is not depicted at scale. Boxplot center lines represent the median, and horizontal lines represent 25% and 75% quartiles, vertical lines represent minimum and maximum values. Letters above boxplots represent the results from Tukey post hoc tests between treatments (see "Results" for more details)
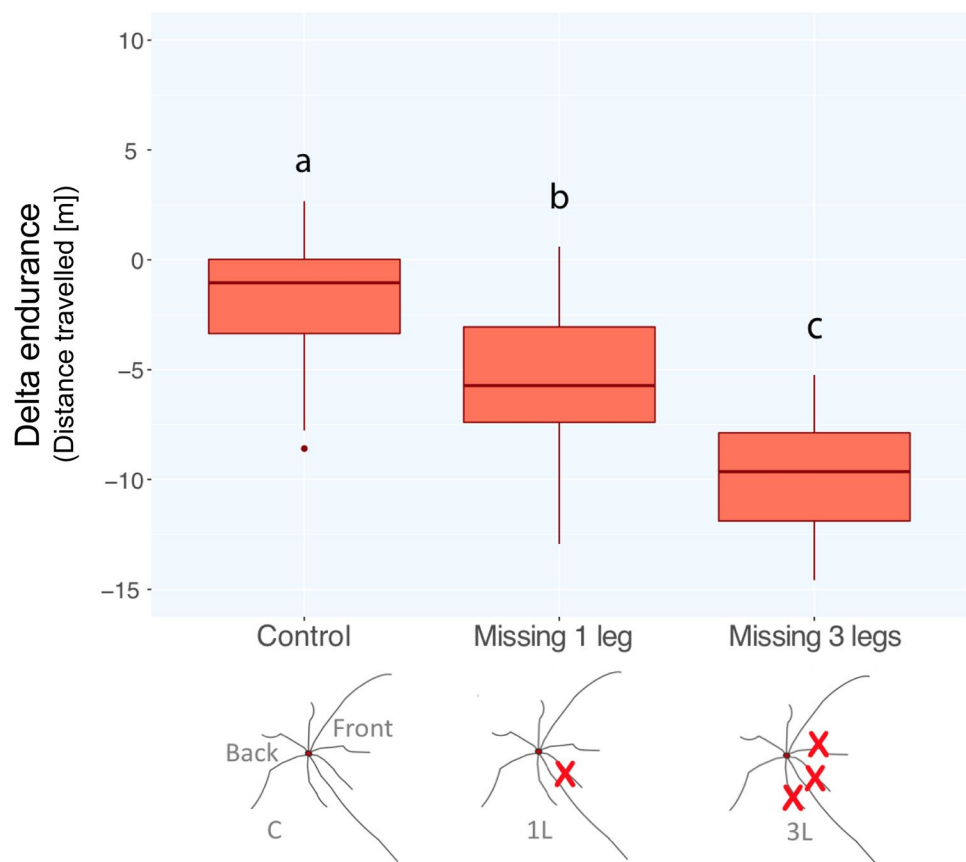
novel in that we provide an experimental demonstration that varying levels of bodily damage affect endurance differently.

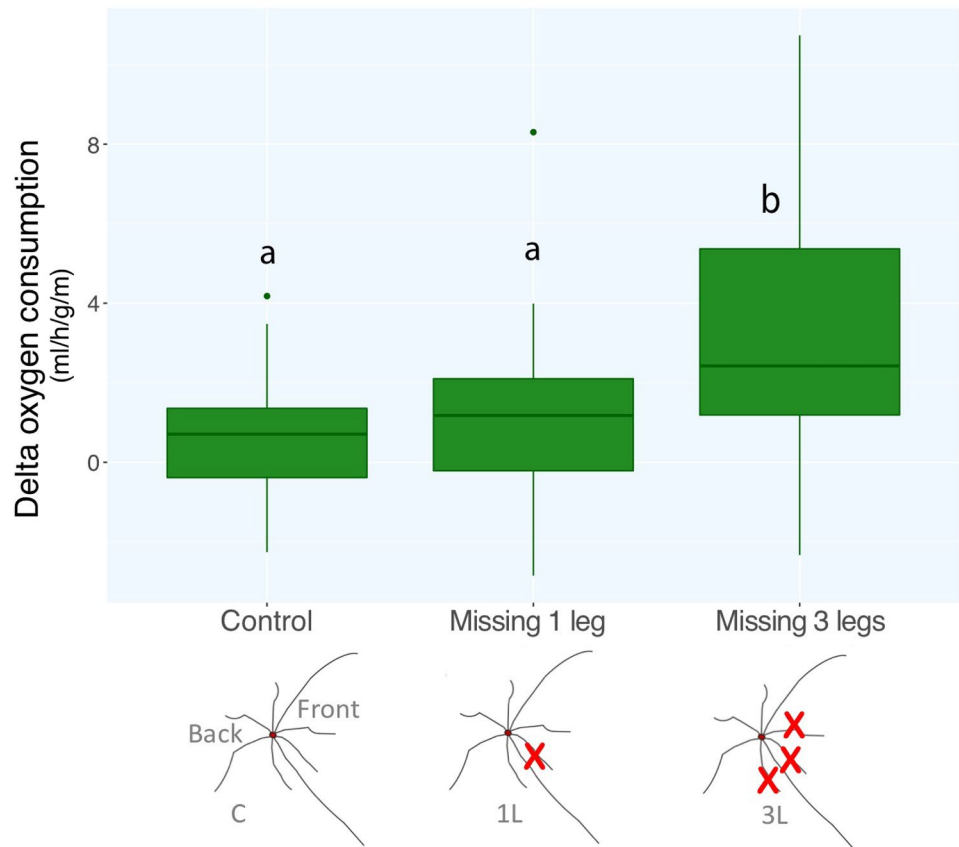## Effects on metabolic costs of locomotion

Only harvestmen that lost three legs experienced an increase in oxygen consumption, whereas individuals that lost one leg were unaffected. This novel finding highlights that consequences on the energetics of locomotion vary with increasing levels of bodily damage. Increased energetic demands after autotomy have been reported for crickets that lost legs (Fleming and Bateman 2007) and geckos that lost tails (Fleming et al. 2009), suggesting that increased metabolic rates may be a general cost of autotomy. For harvestmen, an increase in energetic costs of locomotion after leg loss could be explained by the fact that individuals missing legs not only have fewer muscles, but also fewer spiracles to capture and direct oxygen through the tracheae (Höfer et al. 2000; Schmitz and Perry 2002). This may be particularly critical given that the tracheal system in harvestmen has low diffusion capacities (Schmitz and Perry 2002). Consequently, individuals missing legs might have a higher demand for oxygen and a potential reduction in overall muscular capabilities (Lighton 2002; Sensenig and Shultz 2003; Shultz and Pinto-da-Rocha 2007). The increased oxygen consumption

observed here did not result in more distanced travelled, as endurance actually decreased. We suggest that moving with a reduced number of legs imposed physiological stress, which contributed to increased oxygen consumption. On top of that, these harvestmen had ~40% less legs, which increased the proportional weight and torque requirements to each of the remaining legs. Ultimately, our study shows that when an organism has fewer legs to produce movement, they experience a more energetically challenged condition.

Interestingly, we recorded an effect on oxygen consumption only for animals that had lost three legs, but not one leg. This suggests a threshold of bodily damage (see below) before experiencing costs. Missing one leg likely does not interfere with the overall oxygen capture and distribution, whereas missing three legs does.

Intraspecific variation in body size (but not leg length) affected qualitative aspects of movement in our study animals. Among harvestmen, body size is negatively correlated with resting metabolic rate (O'Brien et al. 2019; Powell 2020), but positively associated with VO$_2$ (Anderson 1993). We found negative correlations of body area with both measures. Smaller individuals had a greater metabolic cost on movement, and fewer legs may exacerbated this energetic challenge by reducing transmission of oxygen to tissues while also imposing greater costs on movement due

Fig. 4 Change in oxygen consumption of harvestmen (*Nelima paessleri*) based on their experimental leg condition while moving on a treadmill inside a sealed open-flow respirometry chamber. The *y*-axis represents the difference between the trial before and the trial after leg loss for the same individual (*n* = 20 individuals per treatment). Diagram in the bottom row depicts each treatment: [intact (C), missing one locomotor leg (1L), missing three locomotor legs (3L)]. Leg length in the diagrams is not depicted at scale. Boxplot center lines represent the median, and horizontal lines represent 25% and 75% quartiles, vertical lines represent minimum and maximum values. Letters above boxplots represent the results from post hoc Tukey tests between treatments (see "Results" for more details)

to disruption of kinematics. In other words, after autotomy, smaller individuals experience greater metabolic costs of locomotion.

Resting metabolic rates (RMR) did not change after autotomy for *N. paessleri* harvestmen. This is consistent with the previous findings in crickets (Fleming and Bateman 2007), geckos (Starostová et al. 2017), and some lizards (Fleming et al. 2009). However, increases in the RMR after autotomy have been found in some lizards (Naya et al. 2007), fiddler crabs (Allen and Levinton 2007), and *Heliconia* bugs (Somjee et al. 2018). At this point, understanding why our findings are similar to some taxa but not others require more work across taxa, body sizes and life histories. However, our findings show that autotomy does not impact the physiological state at resting for harvestmen.

Methodological limitations

Our design is limited in that one of the leg conditions tested in this experiment (3L) was not found in the field. Additionally, we did not test for the effect of removing sensory legs, which were commonly missing in animals in the field. Despite these limitations, we consider our findings informative on the consequences of losing locomotor legs and selection on compensatory mechanisms. Regardless of this, future

work can test the effects of losing sensory legs, locomotor legs from pair IV, and/or other leg conditions found in the field to provide a more comprehensive picture of the energetics of locomotion and leg loss.

The moving behavior of harvestmen changes after autotomy. Leg loss affects the kinematics of different gaits used by *Prionostemma* harvestmen (Escalante et al. 2019, 2020). Those gaits are also used by *N. paessleri* (running and walking). In this experiment, we noted that after leg loss for both 1L and 3L treatments, harvestmen used their legs II to run and walk, similar to *Prionostemma* harvestmen (Escalante et al. 2020). However, variation in endurance and oxygen consumption for *N. paessleri* when using sensory legs to move, and the variation between different gaits is beyond the scope—and experimental design—of this project. Future research can address these topics.

Ecological and evolutionary implications

The patterns which we observed in this study have important life-history implications. First, the inability to sustain locomotion (endurance) could impair the animal's ability to continue moving away from potential predators. Second, increased oxygen consumption is a sign of a more energetically challenging situation for these arachnids. This may

make movement more costly, thereby impairing the animal's overall locomotor energetics, and its physiological condition. This can bring trailing consequences on other processes such as digestion, sensory perception, and/or general foraging behavior.

Sclerosomatidae harvestmen are primarily active at night, continuously moving while foraging for food and searching for mates on the foliage or on the ground (Wade et al. 2011; Proud et al. 2012). During the day, harvestmen species, including *N. paessleri*, remain motionless in roosting places, where they can be found by potential predators (Grether and Donaldson 2007; Grether et al. 2014). Hence, harvestmen will likely have encounters with potential predators such as reptiles, amphibians, and arthropods (Cokendolpher and Mitov 2007) during both nighttime and daytime. When confronted by a predator, harvestmen need to escape quickly. For animals that lost one leg, they would attempt to escape, but their reduced endurance might decrease their likelihood to escape. Harvestmen that lost three legs might be even less able to successfully escape. This because of both their—even greatly—reduced endurance, and a more challenging physiological state due to their increased oxygen consumption.

Potential compensation for leg loss

Harvestmen showed robustness to bodily damage, as individuals missing one leg were able to maintain the same levels of oxygen consumption after autotomy. Our results suggest that there is a threshold for damage before suffering any detectable costs. After surpassing this threshold, individuals could compensate with behavior and/or kinematics to ameliorate the consequences of leg loss. For instance, after leg autotomy *Prionostemma* harvestmen changed kinematic features such as body height, duty factor, type of gait, and temporal leg arrangement while moving (Escalante et al. 2020). These kinematic changes occurred in the same period as the recovery of locomotor performance, particularly velocity and acceleration (Escalante et al. 2020). It is worth noting that such changes occurred to animals that lost 3 legs or 2 legs on the same side, but not when they lost one leg or two legs, one on each side. That finding is consistent with the results presented here. However, if such locomotor recovery correlates with changes in endurance or metabolic costs of locomotion is currently unknown. A follow-up experiment to test for potential compensation on the energetics of locomotion could be done by controlling the time of leg loss and monitoring endurance and energetics over time (Emberts et al. 2019).

Naturally occurring autotomy was common in this group at our field site in California, USA, where we collected the *N. paessleri* harvestmen. One in four individuals was missing one leg. This suggests that there may be selection on strategies to cope with the high probability of losing one leg and could explain the robustness in oxygen consumption of 1L harvestmen. However, the loss of more than one leg was rare, and we did not find individuals that had lost three legs. The survival rates of animals missing more than one leg are likely lower (reviewed in Emberts et al. 2019). Alternatively, it could be rare that the same individual has several encounters with predators. Regardless of the cause, there might be strong selection against losing multiple legs. Consequently, the low frequency of harvestmen missing more than one leg might explain our finding of increased oxygen consumption for 3L individuals, a somewhat unnatural leg condition for this species. This suggests that high levels of leg loss surpass the threshold of bodily damage that these harvestmen are able to compensate for (Escalante et al. 2020).

Long-term compensation in the form of behavioral changes might occur after leg loss. Since individuals can no longer engage in extended physical activity, they might switch to using different defensive strategies (Emberts et al. 2019). Given that harvestmen do not regenerate legs, after autotomy they could deal with potential predators by hiding, becoming more cryptic, jumping, and/or using chemical defenses (Machado and Pomini 2008; Pomini et al. 2010; Chelini and Machado 2014). Additional long-term strategies found in other taxa include modifications in food resource use (Joseph et al. 2018), changes in biomechanics (Herreid et al. 1981a,b; Full and Tullis 1990), limping (Wilshin et al. 2018), using "extra" appendages to replace missing legs (Escalante et al. 2020), or becoming more stationary (Fleming and Bateman 2007).

Conclusions

We found that endurance and the metabolic costs of locomotion in harvestmen are affected by the loss of legs, although the impact of leg loss differed as a function of the magnitude of loss. On one hand, endurance decreased gradually with an increased number of legs lost. On the other hand, oxygen consumption changed (increased) only for harvestmen that lost three legs. The different effects on these two metrics might have different ecological and evolutionary implications. Reduced endurance may impair an animal's ability to continue moving away from potential predators, while increased oxygen consumption makes movement more costly and compromises the animal's overall physiological state. Broadly, our results suggest that loss of legs imposes behavioral and physiological challenges. Our findings contribute to understanding how locomotion is affected by bodily damage, and more broadly, the strategies that animals use to withstand environmental stressors.

Compliance with ethical standards

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