

## SHORT COMMUNICATION

**Metabolism in *Micrathena gracilis*, a stridulating orb-weaver (Araneae: Araneidae)**

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**Abstract.** Metabolism powers all of life's processes, making it fundamental to understanding organisms in nature. For many arachnids, however, we do not have direct measurements of either metabolic rate or the relationship between metabolic rate and body size (i.e., allometric scaling, an important determinant of metabolic rate). Here, we present the first measurements of metabolic rate and allometric scaling in a stridulating spined micrathena spider, *Micrathena gracilis* (Walckenaer, 1805). Since intraspecific variation in metabolic rate may provide insights into variation in energetically costly behaviors, we additionally explore the link between stridulation and metabolism in *M. gracilis*. Our data suggests a link between stridulatory behavior and allometric scaling, such that increased stridulation is associated with a weaker connection between body mass and metabolic rate. We discuss how links between energetics, expression of behavior, and body size inform our understanding of trait variation in these spiders.

**Keywords:** behavior, allometry, respirometry

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Metabolism is the fundamental process that turns energy from the environment into energy used within organisms, powering growth, reproduction, and behavior (Brown et al. 2004). Because of this, metabolic rate can provide insight into larger-scale processes such as development, food web structures, and population dynamics (Kleiber 1961; West et al. 2001; DeLong & Hanson 2009; O'Connor et al. 2009; Burton et al. 2011). Metabolic rate is primarily determined by body size, scaling with a power law function:

$$R = R_0 M^\alpha \quad \text{Equation 1}$$

where  $R$  is metabolic rate,  $R_0$  is normalized metabolic rate,  $M$  is body mass, and  $\alpha$  is the allometric scaling constant. Although the value of  $\alpha$  was historically thought to be approximately 0.75 (Kleiber 1932), recent work has clarified that  $\alpha$  depends on taxonomic identity (Glazier 2006; White et al. 2006; DeLong et al. 2010). However, despite being largely predictable given taxonomic identity and body size, metabolic rates vary even for similarly-sized individuals of the same species (Burton et al. 2011; Uiterwaal & DeLong 2019).

Metabolic rates – or the extent to which certain traits affect metabolism – have not been measured for many species, including the spined micrathena spider, *Micrathena gracilis* (Walckenaer, 1805). Female *M. gracilis* have sclerotized and spined opisthosomas, which are also disproportionately large for their body size compared to other spiders (Levi 1978). Opisthosoma size is linked to metabolic rate and often used in metrics of body condition in other spiders (Uiterwaal & DeLong 2019), although the extent to which this is true for spiders with diverse morphologies is unclear. Here, we measured metabolism in *M. gracilis*, providing estimates of metabolic rate and the allometric scaling of metabolism in this species, and test whether metabolic rate in these spiders is linked to opisthosoma size.

In addition to exaggerated opisthosoma morphology, *M. gracilis* spiders also stridulate in an antipredator context, a behavior where spiders produce vibrations and airborne sound by rapidly flicking their cephalothorax up and down (Hinton & Wilson 1970; Corey & Hebets 2020). *Micrathena gracilis* shows considerable between-individual variation in stridulatory behavior, and individual stridulatory behavior is repeatable across simulated predator attacks in laboratory assays (though more data are needed over longer time scales under natural conditions) (Corey & Hebets 2020). The reasons

for this variation are unclear, since stridulation is not known to be linked to body mass, size, or condition (Corey & Hebets 2020). Because trait evolution is often predicted to be affected by resource allocation trade-offs (Nonacs & Blumstein 2010; Stoddard & Salazar 2011; Wang et al. 2019) and stridulation in other animals is energetically costly (Prestwich 1994; Hack 1997), metabolic rate could help explain stridulatory behavior variation in *M. gracilis*. We therefore further explore the extent to which intraspecific variation in metabolism is linked to stridulatory behavior.

We collected adult female *M. gracilis* ( $n = 54$ ) from Wilderness Park in Lincoln, Nebraska in September 2019 and took all measurements from live spiders on the same day as collection. To record stridulation in response to simulated predator attack, we held each spider by the abdomen (between the observer's thumb and index finger) for ten seconds, applying only enough pressure to hold the spider in place (Corey & Hebets 2020). Many spiders did not stridulate, or only stridulated once, so we therefore treated stridulation as a categorical response with three levels (i.e., whether the spider stridulated zero, one, or multiple times). We then weighed each spider to the nearest ten-thousandth of a gram using an electric scale (Ohaus Adventurer Pro). We photographed the ventral side of individuals next to a size standard, using a Leica MZ16 microscope with an AmScope LED-8WD light source and a Diagnostic Instruments 14.2 64 Mp Shifting Pixel camera, connected to SPOT software (version 5.2). Using ImageJ (Schneider et al. 2012), we measured the spider from the most posterior point along the midline of the ventral opisthosoma to where the midline meets the pedicel, taking the mean of three measurements per spider to obtain ventral opisthosoma length (OLV).

Two to three hours later, we measured resting oxygen consumption as a proxy for metabolic rate using a Presens SDR respirometer (PreSens Precision Sensing, Regensburg, Germany). We placed each spider into a 2mL respirometry vial and randomly assigned vials to positions in six-by-four slot Presens Sensor Dish Reader (SDR) respirometry trays. In each tray, we also included two or three control vials containing only air. We used a total of four trays, running two trays at a time. To minimize measurement error in the light-sensitive system and reduce disturbance to the spiders, we wrapped all trays in aluminum foil. We took respirometry measurements for one hour at room temperature and pressure (approx. 23°C and 975 mbar, respectively).

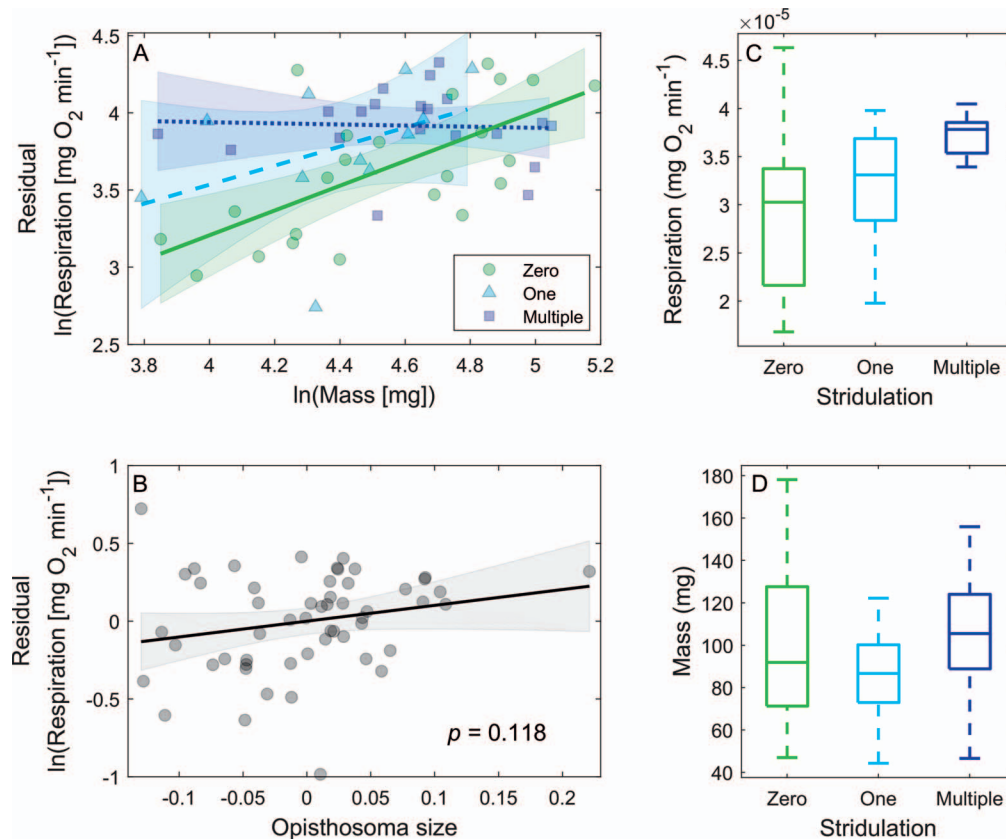


Figure 1.—Respiration and stridulation in *M. gracilis*. Partial residual plot of (A) body mass and stridulation (Solid line: Zero stridulations. Dashed: One. Dotted: Multiple) and (B) opisthosoma size (opisthosoma length-body size residuals) on respiration rate in the best-performing model. Shaded areas represent confidence intervals. (C) Fitted respiration rates and (D) body mass of spiders in each stridulation category.

After respirometry measurements, we calculated the volume of individual spiders by freezing half of the 54 spiders at  $-20^{\circ}\text{C}$  before dropping spiders individually into water-filled 10 mL graduated cylinders, and recording the water displacement (Radtke et al. 2006; Uiterwaal & DeLong 2019). The remaining spiders were released into the field near their collection locations. To calculate the volumes of these spiders, we used the released spiders' masses and the mean density (mass/volume) of the sacrificed spiders.

To calculate respiration rates, we first discarded the initial 20 minutes of each spider's oxygen trace to eliminate effects of acclimation or system equilibration. We then obtained a slope of oxygen concentration over time using ordinary least-squares regression. For each tray, we calculated the mean slope of empty vials and subtracted this value from the spider slopes to account for any background changes in oxygen concentration. Due to a conversion error when entering air pressure into the respirometry software, respirometry data from two of the SDR trays was systematically different from trays for which air pressure was entered correctly. To correct the data from those two trays, we calculated the z-score of the respiration rate for each spider with incorrectly recorded data. We multiplied these z-scores by the standard deviation of the correctly recorded respirometry rates and added this to the mean respiration rate of the correctly recorded rates to obtain what the respirometry data would have been if the air pressure had been entered correctly.

Differences in spider body volume resulted in differing initial amounts of oxygen available to each spider in the respirometry vials. To account for this, we subtracted spider volume from vial volume (2.87 ml) to obtain the volume of air available to each spider. Using this value, we converted oxygen consumption from  $\text{ppm min}^{-1}$  ( $\text{g l}^{-1} \text{min}^{-1}$ ) to  $\text{g min}^{-1}$ , giving an absolute respiration rate for each individual.

We log-transformed respiration, body mass, and OLV because these measurements often have power law relations, such that metabolic rate scales nonlinearly with body size measurements. Because OLV is likely to vary with body mass, we then used residuals from an OLV to body mass regression as our measurement of opisthosoma size (indicative of opisthosoma length relative to body size; Corey & Hebets 2020). We fit linear models to the data, using metabolic rate as the dependent variable and all combinations of body mass, stridulation category, and opisthosoma size as predictor variables. We also included all possible two-way interactions between predictors in our model set. We selected as the best-performing model the one with the lowest Akaike information criterion (AIC) value (Burnham & Anderson 2004). We used Matlab R2018b (MathWorks) for all statistical analyses.

The mean weight of individual spiders was  $98.8 \text{ mg}$  ( $\pm 4.3 \text{ SE}$ ) with a mean density of  $0.74 \text{ g/ml}$  ( $\pm 0.04 \text{ SE}$ ). The mean respiration rate was  $3.42 \times 10^{-5} \text{ mg O}_2 \text{ min}^{-1}$  ( $\text{VO}_2$ :  $14.7 \mu\text{l O}_2 \text{ h}^{-1} \text{ g}^{-1}$ ). Twenty-three spiders never stridulated, 11 spiders stridulated once, and 20 spiders stridulated multiple times. Body mass and OLV were significantly correlated (see Fig. S1, online at <https://doi.org/10.1636/JoA-S-21-005.s1>). There was no difference in body size across stridulation categories ( $F(2,51) = 1.84$ ,  $P = 0.170$ ; Fig. 1D). The best-performing model contained opisthosoma size and an interaction between stridulation category and body mass (Table 1), but this model did not perform significantly better than the next best model, which contained only the interaction between stridulation and body mass ( $\Delta\text{AIC} = 0.47$ , see Table S1, online at <https://doi.org/10.1636/JoA-S-21-005.s1>).

In the best model, respiration rate increased with body mass to a power of 0.80 (i.e., the allometric scaling exponent) ( $\text{SE } 0.18$ ;  $P <$

Table 1.—Results from the best-supported linear model (using AIC model selection) for metabolic rate.

Term	Estimate	SE	<i>t</i>	<i>p</i> value
Intercept	-14.12	0.84	-16.84	<0.001
ln(Mass [mg])	0.80	0.18	4.36	<0.001
Stridulation (One)	1.07	1.67	0.64	0.526
Stridulation (Multiple)	4.08	1.35	3.02	0.004
Opisthosoma size	1.02	0.64	1.59	0.118
ln(Mass):Stridulation (One)	-0.19	0.38	-0.49	0.624
ln(Mass):Stridulation (Multiple)	-0.84	0.29	-2.85	0.006

0.001). Spiders that stridulated multiple times had higher respiration rates than those that never stridulated ( $P = 0.004$ ). There was no difference in respiration rates between spiders that stridulated once and those that never stridulated ( $P = 0.526$ ) or stridulated multiple times ( $P = 0.096$ ). There was an interaction between stridulation and mass, such that the effect of mass on respiration decreased with increased stridulation ( $P = 0.006$ ) (Fig. 1A). There was no significant effect of opisthosoma size on respiration ( $P = 0.118$ ) (Fig. 1B), although model performance improved marginally with inclusion of this term. The direction and significance of the effects of mass, stridulation, and the interaction term were the same in the top model and the second-best model.

Our results for female *M. gracilis* are the first measurement of the allometric scaling of metabolism in spiny orb weavers and the first measurement of metabolic rate in *M. gracilis*. The mean mass-specific metabolic rate of  $14.7 \mu\text{l O}_2 \text{ h}^{-1} \text{ g}^{-1}$  is lower than that of many other spider species, but within the reported range for spiders ( $13\text{--}356 \mu\text{l O}_2 \text{ h}^{-1} \text{ g}^{-1}$ ) (Anderson 1970; Anderson & Prestwich 1982). We suspect this may be due to the relatively large amount of non-living tissue on the opisthosoma, which may significantly increase body mass without contributing to resting metabolic demands. Our allometric scaling estimate of 0.80 is comparable to those recorded for other spiders, which typically fall between 0.7 and 1.0, including 0.74 for theridiids (Anderson 1994), 0.85 for lycosids (Uiterwaal & DeLong 2019), and a mean estimate for all spiders of 0.80 to 0.82 (Anderson & Prestwich 1982; Terblanche et al. 2004). Other orb weaving spiders have allometric scaling exponents slightly higher than our estimate for *M. gracilis*, with reports ranging from 0.86 (Kawamoto et al. 2011) to 0.96 (Anderson & Prestwich 1982). As in other spiders, the scaling exponent for *M. gracilis* is not significantly different from the theoretical prediction of 0.75 (Kleiber 1932).

However, we found that allometric scaling differed depending on stridulatory behavior. We found scaling estimates of 0.80 for non-stridulating spiders, 0.61 for spiders that stridulated once, and indistinguishable from 0 for spiders that stridulated multiple times (Fig. 1A). Thus, the link between metabolic rate and stridulation weakened as body mass increased, such that respiration rates for the largest spiders were almost indistinguishable from each other regardless of stridulatory behavior. Similarly, respiration rates for spiders that stridulated multiple times were comparable regardless of body size (Fig. 1A). The lower scaling constants of stridulating spiders fall well below those previously reported for spiders (Anderson & Prestwich 1982).

Overall, the spiders that stridulated the most had the highest metabolic rates (Figs. 1A, C), although these results are largely driven by substantial differences in the metabolic rates of the smallest spiders across stridulation categories (Fig. 1A). This finding is consistent with metabolic rate increases as a result of stress or activity (Carey 1979; Sloman et al. 2000), including stridulation in insects (Stevens & Josephson 1977; Mowles 2014). However, we cannot disentangle whether stridulation elevates metabolic rates or whether existing elevated metabolic rates facilitate stridulation, as the closed system required for respirometry precluded us from encouraging stridulation via simulated

predator attacks during our metabolic rate measurements. The observed variation in metabolic rate could thus be a plastic response to stress, a trait with ramifications for signaling behavior, or both.

Increases in metabolic rate due to stridulation are well-documented in other animals (Mowles 2014, crickets; Stevens & Josephson 1977, katydids), so we might expect our results to reflect increases in metabolic rate as a result of stridulation. If the observed effects of mass and stridulation on metabolic rates are indeed due to mass-dependent metabolic costs of stridulation, this could manifest in several ways. Individuals that stridulate could face an immediate cost, the magnitude of which depends on body size. Alternatively, the immediate costs of stridulation may be mass-independent, but smaller spiders may experience exaggerated recovery times, allowing the largest spiders to recover while the metabolic rates of the smallest spiders still reflect the effects of stridulation. Lastly, it is possible that stridulation does not affect metabolic rate; stridulation could instead depend on metabolic rate such that only the individuals with the highest metabolic rates have “access” to this behavior. Thus, there may be a threshold metabolic rate that must be met for an individual spider to be capable of stridulating.

Because energetically costly behavior can impose trade-offs (Bennett et al. 2013; Harrison & Preisser 2016; Khater et al. 2016), the size-dependent link between metabolic rate and stridulation suggests that stridulatory behavior could change with body size in *M. gracilis*. That is, if stridulation is costlier for smaller spiders (e.g., due to larger increases in metabolic rate or longer recovery times) or easier for larger spiders (e.g., due to a threshold metabolic rate that is easier for larger spiders to reach), larger spiders should stridulate more frequently. Despite these expectations, our results confirm previous findings that there is no relationship between *M. gracilis* stridulatory behavior and body size (Corey & Hebets 2020).

Opisthosoma size is linked to metabolic rates in other spiders, as the relatively flexible opisthosoma may expand or contract in response to food intake, reproductive status, or other factors that may influence metabolic rate (Uiterwaal & DeLong 2019). Opisthosoma size did not significantly influence metabolic rate in *M. gracilis*, and we suggest that the sclerotized opisthosoma cuticle and spines may help explain this. In female *M. gracilis*, the dorsalmost region of the hardened cuticle does not expand or contract in response to food consumption or reproductive status (Corey pers. obs.). Variation in opisthosoma size is likely affected by the amount of cuticle (i.e., non-living chitinous exoskeleton) in addition to variation in the amount of metabolically active tissues. We also do not know if production of this cuticle – and the potential energetic costs of movement associated with thicker cuticle and/or spines – are linked to metabolic rate. A potential relationship between metabolic rate and opisthosoma size in *M. gracilis* may therefore be impacted by multiple sources. Comparing metabolic rates across spiny orb-weaving spider species could shed more light on the extent to which individual foraging and reproductive status, opisthosoma spines, and metabolic rates are connected. Phylogenetic studies suggest that elongated opisthosoma spines have evolved multiple times within the genus *Micrathena* Sundevall, 1833 (Magalhães & Santos 2012) – comparative studies of metabolic rate may therefore inform our understanding of spine number and size variation across *Micrathena* species.

We note that several additional factors may influence metabolic rate in *Micrathena gracilis*. For instance, *M. gracilis* are less likely to stridulate when collected off their webs towards the end of their reproductive season (late September at our field site, Corey pers. obs.). We hypothesize that this change in stridulatory behavior could be due to senescence and/or a seasonal decline in temperature. Thus, temperature and individual age may additionally influence metabolic rates and the relationship between metabolic rates and stridulation in this species. Additionally, because *M. gracilis* males are much smaller than females, metabolism in males is likely to differ from the results we report here for females.

Because mass and metabolic rates are associated with population size, foraging, and reproduction, exploring factors that modify the relationship between mass and metabolism informs our understanding of how ecological communities are structured (DeLong & Hanson 2009; Marañón 2015). Here, we present estimates of metabolic rate and the allometric scaling of metabolism in a geographically widespread spider species (Levi 1978), and further show that mass, metabolism, and stridulation are linked. Our study continues to build a foundation for understanding the metabolism of organisms in nature and clarifying how energetics, expression of behavior, and body size can be connected.

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#### SUPPLEMENTAL MATERIALS

Figure S1.—Correlation between opisthosoma length and mass.  
Table S1.—Models for respiration ranked by AIC.  
Online at <https://doi.org/10.1636/JoA-S-21-005.s1>

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