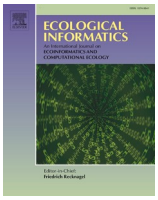
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The bias of the biomass - different methods to determine spider biomass in ecological samples

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ABSTRACT

Biomass is an important functional trait in ecology, because body size and mass influence biological parameters such as activity, energy demand, metabolic rates, and consequently population and food-web dynamics. In arthropod monitoring, biomass is often used as a surrogate for abundance to detect population trends across large spatial and temporal scales. However, arthropods are typically trapped in a preservative, preventing direct measurement of fresh mass. Consequently, biomass is often estimated using drained weight, although preservation fluids may affect biomass estimates and body size-mass regressions may provide more accurate results. To assess the accuracy and variability of biomass estimation methods for epigeic spiders, we compared the fresh mass of freshly captured spider samples with their drained weight and biomass estimates based on body size-mass regression models from the literature. Regression models used body length and width measured for each spider or species-specific mean body length from a public database. Regression estimates generally performed better than drained weight when compared to fresh mass. Among the tested regression models, the combination of measured body length and width showed high accuracy and the lowest variability. Drained weight overestimated sample biomass by approximately 48% and showed high variability. Using species-specific mean body lengths from a public database resulted in the highest accuracy relative to fresh mass but also high variability. Our findings highlight the importance of considering required accuracy, variability, and methodological effort when selecting biomass estimation methods for ecological monitoring.

1. Introduction

Trait-based approaches are increasingly used in ecology to understand structure and function of species assemblages of terrestrial arthropods and to assess the consequences for ecosystem processes and changes (Müller et al., 2022; Wong et al., 2018). These approaches strongly rely on standardized measurements of the traits to enable analyses across taxa, habitat types and regions (Moretti et al., 2017). Biomass is one essential functional trait of species. Body size and mass, which are strongly correlated, influence many biological functions such as activity, nutrient requirements, metabolic rates, energy fluxes, population and food web dynamics, as well as predator-prey interactions (Hódar, 1996; Sohlström et al., 2018; Vucic-Pestic et al., 2010). This also applies to spiders, where body size and biomass were used in several ecological studies to evaluate the niche filtering mechanism of environmental gradients such as temperature and humidity (Entling et al., 2009) or as an indicator for environmental concerns (Kobel-Lamparski and Lamparski, 2021).

In recent years, biomass of bulk samples received considerable attention as a surrogate for abundance in long-term biodiversity monitoring of insect populations. The disturbing report of Hallmann et al. (2017) on a more than 75% decline in total flying insect biomass in protected areas over 27 years provoked a worldwide debate on insect decline and triggered various additional studies (Dalton et al., 2023; Hallmann et al., 2021; Hallmann et al., 2025; Leather, 2017; Müller et al., 2023; Shirey et al., 2026; Uhler et al., 2021; Wildermuth et al., 2025). With this background, standardized assessment of insect populations was widely demanded (e.g. Cardoso and Leather, 2019). As a

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consequence, a state-wide insect monitoring was initiated in the German federal state of Baden-Wuerttemberg (BW) and since 2018 implemented by the State Agency for the Environment Baden-Wuerttemberg (LUBW, 2026; Oellers et al., 2022). Various taxa and indicators are examined annually through different programs at a large number of locations in different habitat types. The program "Epigeic Biomass" covers 78 agricultural sites that are regularly sampled with pitfall traps and species diversity, abundance and biomass of ground beetles (Carabidae) are evaluated. For the bycatch, i.e. spiders, staphylinids and remaining arthropods only individuals are counted and the biomass is measured at the sample level as drained weight (LUBW, 2026; Oellers et al., 2022).

Measuring the biomass of arthropods by directly weighing individual specimens requires logistic effort, is labour intensive, and for ecological samples of arthropods with usually high abundances nearly impossible. In particular, to determine the fresh mass, individual animals must also be anesthetized or killed and weighed promptly with a high precision balance (Penell et al., 2018; Sohlström et al., 2018). Drying spiders for the determination of biomass (dry mass) is generally not recommended, when the specimens are to be identified afterwards and kept as vouchers in collections, because their legs break too easily after being dried (BfN, 2023) and species identification in general becomes more difficult. Even more relevant, in most ecological surveys, arthropods are already preserved with a fluid in traps, making it impossible to determine their fresh mass by weighing. For this reason, the biomass of individual or combined samples is often determined by draining the liquid in a standardized procedure before weighing with a precision scale. This has already been proposed by BfN (2023) for the insect monitoring in Germany (referring to a standard procedure described by Ssymank et al., 2018) and followed in the statewide insect monitoring program in Baden-Wuerttemberg. Any weight measurement of preserved arthropods will differ from the real biomass of the living specimens due to the penetration and adhesion of the preservative liquid to the body, particularly pronounced in spiders due to the soft cuticle of the opisthosoma and their hairiness. This is not severe for most research questions as long as the difference is not too big and remains systematic. However, at present, it is unclear how large the discrepancy is between the drained weight and the actual biomass of spiders, especially for samples with many individuals of varying size and how precise the biomass estimation of a monitoring study would need to be. Evidence suggests that trapping and preservation fluids can bias biomass estimates (Braun et al., 2009; Knapp, 2012; Weiss and Linde, 2022) and estimating biomass using body size-mass regressions can yield more accurate results (Weiss and Linde, 2022). Length-mass regressions have been proposed to predict body mass on body length measurements (for arthropods: Gruner, 2003; Sohlström et al., 2018; for spiders: Hódar, 1996; Höfer and Ott, 2009; Penell et al., 2018). However, for such approaches, individuals must be measured with high precision and suitable regression parameters for the taxonomic group and the geographic region are needed.

Some studies showed that prediction accuracy improved when additional predictors were included (e.g. additional body dimensions such as body width, taxonomy, life-history traits, habitat preference) to determine more specific regression models (Penell et al., 2018; Sohlström et al., 2018) Further, applying regression models from (spider) samples of one climatic region to samples of another climatic region results in reduced precision (Höfer and Ott, 2009). This, in turn, supports Hódar's (1996, p. 430) conclusion that the choice of the regression model used depends on the aim of the study and the associated required accuracy, and that the "consequence of a careless application of estimates seriously weakens the results of the research". Hence, with regard to the "Epigeic Biomass" monitoring of BW, and especially the spiders sampled therein, we used and tested the taxon-, habitat- and geographically specific regression models from Penell et al. (2018) for their accuracy. To determine biomass of samples in a monitoring program, measuring each individual is already a considerable effort. Estimating biomass by regression seems feasible, when the same regression parameters are applicable to all individuals of a higher taxon in a sample, without the need to first identify the individuals to a lower level (i.e. family, genus or species) to use specific regression parameters. If samples were already identified to the species level, biomass could be estimated by regressions using species-specific mean body lengths if available from literature or trait databases (e.g. ARAMOB, 2026; Nentwig et al., 2026).

Most studies we are aware of generated regression models and evaluated their goodness-of-fit but do not validate their performance against a validation dataset. Our research revealed only a few studies in which this was the case. Two of them performed a validation against dry mass (Rogers et al., 1976; Sample et al., 1993), and one study performed a validation against fresh mass (Weiss and Linde, 2022). None of these studies used spiders as the target organism (Supplementary Table S1). To address this research gap and improve the assessment of relevant changes in spider populations during long-term monitoring studies, the following hypotheses were tested: H1) Measuring drained weight is not the most accurate method to determine biomass of spider samples in monitoring programs and is associated with high methodological variability. H2) Estimating the biomass of spider samples using regression models based on body size measurements shows greater accuracy and lower variability than the drained weight. H3) Species-specific mean body length values for spiders from public databases can yield comparably accurate estimates of sample biomass compared to values derived from the measurement of individual specimens.

To test these hypotheses, an experiment was conducted. We determined the absolute and relative deviation of the drained weight and regression estimates, respectively, in comparison to actual fresh mass of samples, i.e. by weighing living spiders. The assessment of alternative methods for analysing biomass of monitoring samples has already been recommended in the BfN (2023) methodological guidelines for biodiversity monitoring programs in Germany. Additionally, we evaluate the deviation between the measured drained weights and regression estimates based on a number of samples that derive directly from the statewide insect monitoring of BW. We also compare the regression estimates of the directly measured body lengths with the estimates using species-specific mean body length values from a public database (ARAMOB, 2026).

Furthermore, we know from other studies in Germany and Switzerland, as well as from the monitoring data from BW, that the ground-dwelling spider fauna captured in pitfall traps (or other activity-based methods) in agricultural habitats is usually dominated by few species, with Lycosidae and Linyphiidae being the two most abundant spider families (e.g. Blick, 1999; Blick et al., 2000; Kitt and Kielhorn, 2021; Plath et al., 2021; Rischen et al., 2021). That leads to an additional hypothesis H4): sorting, counting, identifying and estimating the biomass of the dominant large species (i.e. lycosid species) should, at least in arable land, provide sufficiently accurate biomass estimates per sample to serve as a proxy for total fresh mass.

2. Material and methods

2.1. Sampling and processing spiders for the experiment

For the experiment, 425 spiders were caught alive at 18 different sites in the federal state of Baden-Wuerttemberg and Rhineland-Palatinate from May to September 2025. Spiders were caught by hand or pitfall traps (opening diameter 8 cm, no trapping fluid, emptied daily) and each specimen was put into a separate vial. In order to best represent the permanent agricultural sites covered by the monitoring program, sites with a strong anthropogenic influence were selected for the experiment, i.e., regularly cut grassland (meadows) and arable land. After capturing, the spiders were transferred to the lab, where they were anesthetized with CO_2 and weighed on a precision scale (Sartorius QUINTIX35-1S; Sartorius AG, 37079 Göttingen, Germany) to the nearest 0.001 g (measurement error: 0.00017 g). Each spider was given a unique

label before it underwent the preservation process. Spiders were first stored in propylene glycol (PG) for 2 weeks, then transferred to ethanol 80% and stored for at least four further weeks to ensure that the bodies were completely saturated with the preservative fluid. This imitates a widely used modern sampling protocol in pitfall trap studies and is also applied within the framework of the Epigeic Biomass monitoring program (see Oellers et al., 2022). It is important to note here that we deliberately chose to use PG instead of Renner solution, which is currently used in the Epigeic Biomass monitoring program, because PG is already widely accepted as future trapping fluid of this monitoring program (BfN, 2023) as it also conserves DNA (Höfer et al., 2015; Nakamura et al., 2020). Further, the spiders used for the development of the regression parameters by Penell et al. (2018) were stored in PG. We acknowledge that this decision imposes certain limitations and affects the comparison between the samples from the experiment and the current monitoring scheme. All spiders were identified; adults to species level and juveniles to genus or family level. They were photographed with a KEYENCE VHX-7100 digital microscope (KEYENCE DEUTSCHLAND GmbH, 60540 Frankfurt, Germany) and measured with the built-in measurement function to the nearest 0.01 mm (Fig. 1). Prosoma and opisthosoma length were measured individually and both parameters were summed up, resulting in the full body length.

Based on the body size measurements, the mass of each spider was calculated using regression models. Additionally, the mass of each adult spider was estimated by regressions using the mean body length of females and males for each species taken from a public database (ARAMOB, 2026). Juvenile spiders were classified into the two developmental stages "juvenile" and "subadult". For each taxon and developmental stage, mean body length was determined. If multiple individuals per group were available, the mean was calculated based on individual measurements. If only a single individual was present, its measured body length was used as the mean.

2.2. Regression models

Seven different regression models were used to estimate spider biomass from body size measurements. Six based on Penell et al. (2018) and one on the data from the experiment (Table 1). Individual body mass was estimated using a log-log linear regression model:

$$\ln(rm_i) = a + b \times \ln(x_i) \quad (1)$$

corresponding to a power function on the original scale:

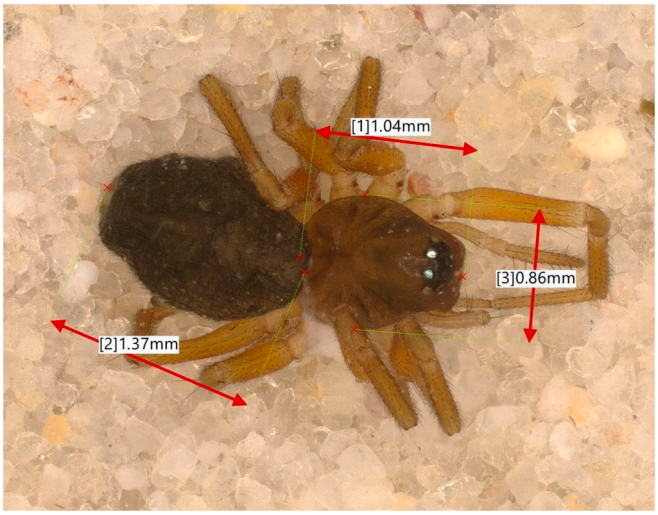


Fig. 1. One female specimen of *Oedothorax apicatus* from the experiment. All spiders were photographed and measured with the KEYENCE VHX-7100 digital microscope.

Table 1
Regression statistics for the seven regression models based on the data from Penell et al. (2018) and the experiment (Own sampling): sample size (N), range of body length, adjusted determination coefficient (adj. R^2), residual standard deviation (σ) and parametric lognormal correction factor (CF). For all regression models $p < 0.001$. BL = body length, PL = prosoma length, PW = prosoma width, gr = habitat specificity for ground spiders, exp. = data based on our experiment.

Dataset/Group	Model	N	BL range [mm]	adj. R^2	σ	CF
Penell et al. (2018) All spiders	BL	189	1.15–16.8	0.959	0.31	1.0492
	BL × PW		1.15–16.8	0.972	0.26	1.0344
	PL		1.15–16.8	0.933	0.39	1.0790
	PW		1.15–16.8	0.928	0.41	1.0877
Penell et al. (2018) Ground spiders	BL_{gr}	105	1.10–12.6	0.981	0.22	1.0245
	BL × PW_{gr}		1.10–12.6	0.977	0.24	1.0292
Own sampling All spiders	BL_{exp}	425	1.29–11.13	0.942	0.30	1.0460

$$rm_i = \exp\left(a + b \times \ln(x_i)\right) \quad (2)$$

where rm_i is the mass of individual i (in mg) estimated by regressions, a is the intercept, b is the scaling exponent (slope), and x_i is the morphological predictor variable (in mm).

Back-transformation of the log-scale predictions yields the median estimated mass on the original scale. The mean estimated mass on the original scale can be obtained by applying a parametric lognormal correction factor:

$$CF = \exp\left(\frac{\sigma^2}{2}\right) \quad (3)$$

where σ is the residual standard deviation of the respective regression model (Table 1). For regression models using species-specific mean body length, the residual standard deviation of the body length regression model was used for the parametric lognormal correction. The corrected estimate of individual body mass is therefore calculated by multiplying the back-transformed estimate by the correction factor:

$$rm_{i,corr} = \exp\left(a + b \times \ln(x_i)\right) \times \exp\left(\frac{\sigma^2}{2}\right) \quad (4)$$

Three different body size measurements were used by Penell et al. (2018): body length (BL_i, mm), prosoma length (PL_i, mm) and prosoma width (PW_i, mm). The fourth body measurement is a composite predictor defined as the product of body length and prosoma width. The predictor is defined as $x_i = BL_i \times PW_i$ (in mm^2), leading to:

$$rm_{i,corr} = \exp\left(a + b^{*}\ln(BL_i \times PW_i)\right) \times \exp\left(\frac{\sigma^2}{2}\right) \quad (5)$$

For BL and BL × PW we applied two sets of their parameters, one for "all spiders" and one habitat-specific for "ground spiders" (BL_{gr}, BL × PW_{gr}). The model based on experimental data only included BL as parameter (BL_{exp}). Additionally, we used species-specific mean body lengths (BL_{mean} $BL_{gr,\,mean}$ and $BL_{exp,\,mean}$ for the experimental data) from a public database (ARAMOB, 2026) to estimate biomass with the corresponding models. In total, this resulted in 10 regression model-body measurement combinations for biomass estimation (Table 2).

2.3. Pseudosample generation and biomass estimation

A total of 425 spiders were randomly assigned to 20 pseudosamples using a custom R script with a fixed seed (123). The objective was to

Table 2
Regression parameters (a = intercept, b = slope) with standard errors (SE) for the seven regression models. BL = body length, PL = prosoma length, PW = prosoma width, gr = habitat specificity for ground spiders, exp. = data based on our experiment. For the regression models using species specific mean body length from a public database, the BL regression parameters are used, respectively.

Dataset/Group	Model	*a*	SE	*b*	SE
Penell et al. (2018)	BL	−1.72389	0.06	2.69638	0.04
All spiders	BL × PW	−0.40055	0.04	1.38032	0.02
	PL	0.47993	0.04	2.51877	0.05
	PW	1.04109	0.04	2.66434	0.05
Penell et al. (2018)	BL_{gr}	−1.86873	0.06	2.80107	0.04
Ground spiders	$BL \times PW_{gr}$	−0.43006	0.05	1.37422	0.02
Own sampling All spiders	BL_{exp}	−1.69536	0.05	2.87308	0.03

generate a broad range of pseudosample sizes (approximately 5–50 spiders per sample) to reflect the variation in sample sizes typically observed in two-week pitfall-trap samples from monitoring programs. Because the initial random assignment resulted in a narrower distribution (5 to 34 spiders per sample), spiders were manually reassigned between pseudosamples. This adjustment expanded the final range to 5–43 individuals per sample while maintaining the 20 pseudosamples and the total sample size of 425 individuals, which was considered to sufficiently reflect typical variations. Total pseudosample mass RM_{sample} (mg) for a given sample containing N individuals was calculated by summing the estimated individual masses:

$$RM_{sample} = \sum_{i=1}^{N} rm_i = \sum_{i=1}^{N} \exp(a + b^{*}\ln(x_i)) \times \exp\left(\frac{\sigma^2}{2}\right) \quad (6)$$

The exact same procedure was followed to calculate total sample fresh mass (FM_i and fm_i). The drained weight of each of these 20 samples was measured following the protocol of the Epigeic Biomass monitoring program as described by Oellers et al. (2022).

The regression parameters from the literature and the experiment were also applied to 81 spider samples from the Epigeic Biomass monitoring program for which we had drained weights and of which all specimens had been identified, in order to further analyse the deviation between drained weight and regression estimates with a bigger sample size.

2.4. Statistical analysis

All statistical analyses were performed using R Version 4.5.2 (R Core Team, 2025). The packages *dplyr* (Wickham et al., 2014a; Version 1.2.1) and *tidyr* (Wickham et al., 2014b; Version 1.3.2) were used to organize the data; the packages *ggplot2* (Wickham, 2016; Version 4.0.3) and *beanplot* (Kampstra, 2008; Version 1.3.1) in combination with Inkscape Version 1.4.3 (Inkscape Project, 2025) were used for data visualization.

Absolute and relative deviations between biomass estimation methods and the fresh mass of the 20 pseudosamples were analysed using linear mixed-effects models with *Method* as a fixed effect and *Sample* as a random effect. The model to analyse the relative deviations additionally included the fresh mass of only the lycosids of a pseudosample. Initial models assumed homogeneous residual variances and were fitted using the *lme4* package (Bates et al., 2015; Version 2.0-1). Inspection of residual diagnostics revealed method-specific heteroscedasticity, indicated by two distinct clusters in residuals versus fitted plots. To account for this variance heterogeneity, we fitted linear mixed-effects models using the function lme() from the *nlme* package (Pinheiro et al., 1999; Version 3.1-168), of the form:

$$Y_{ij} = \beta_0 + \beta_{Method_j} + u_i + \varepsilon_{ij} \quad (7)$$

where Y_{ij} is the deviation for method j in sample i, β_o is the intercept, β_{Method_j} is the fixed effect of *Method*, u_i is the random intercept for sample i and ε_{ij} is the residual error term. The random effects were assumed to follow $u_i \sim N\left(0,\ \sigma^2_{Sample}\right)$, while residual variances were explicitly modeled separately for each method using the varIdent variance structure. The fixed effect of *Method* was tested using an F-test, yielding $F_{9,171} = 31.019$, $p < 0.0001$ for the absolute deviation model and $F_{9,171} = 60.258$, $p < 0.0001$ for the relative deviation model. Nevertheless, the clusters persisted. One cluster comprised the residuals of the regression estimates, whereas the second cluster comprised the residuals of the drained weights. We therefore examined the residuals separately for each method. As no systematic patterns were detected in either the Q-Q plots or the normalized residuals-versus-fitted plots for the individual methods, the model was considered to provide an adequate fit. A likelihood-ratio test comparing the homogeneous and method-specific residual variance models, both fitted using the *nlme* package, confirmed that allowing method-specific residual variances significantly improved model fit (absolute deviation model: LRT = 145.163, df = 9, $p < 0.0001$; relative deviation model: LRT = 186.353, df = 9, $p < 0.0001$). Pairwise post-hoc comparisons between methods were done using estimated marginal means, calculated with the *emmeans* package (Lenth and Piaskowski, 2017; Version 2.0.3). *P*-values were adjusted for multiple testing using Tukey's method to control the family-wise error rate across all pairwise comparisons among the methods. In addition, each method was tested against zero to determine whether its mean deviation differed significantly from fresh mass. The mean relative deviations between drained weight and regression estimates (of all regression model-body measurement combinations) of the 81 monitoring samples were calculated and statistically tested using a linear mixed-effects model with *Method* as a fixed effect and *Sample* as a random effect. The model was fitted using the lme() function from the *nlme* package. No pairwise comparisons between methods were performed. Instead, each method was tested separately against zero using estimated marginal means calculated with the *emmeans* package. Since these tests comprised multiple comparisons using the same reference value, the *p*-values were adjusted using the Bonferroni correction.

2.5. Accuracy and variability of biomass estimates

To evaluate the performance of the drained weight and regression models, we distinguished between accuracy and variability of the estimates. Accuracy was defined as the closeness of an estimated sample biomass to the reference value, i.e. the total fresh mass of all spiders in the respective pseudosample. Accuracy ultimately was assessed using the mean relative deviation from fresh mass, with values closer to zero indicating higher accuracy (lower deviation). A relative deviation of zero therefore indicates an unbiased estimate, whereas positive and negative values indicate over- and underestimation, respectively. Variability was defined as the dispersion of the method-specific relative deviations among the 20 pseudosamples and was quantified using the standard deviation (SD).

3. Results

3.1. Sample composition

A total of 425 spiders (296 adults, 129 juveniles/subadults) of 44 species (Supplementary Table S2) were collected for the experiment, with a total biomass (fresh mass) of 6987.5 mg. The 81 monitoring samples included 1388 spiders (only adults were measured) from 47 species. Lycosidae (wolf spiders) dominated the samples for the experiment by individuals and even stronger by their biomass, matching the composition of the monitoring samples (Table 3). Of the 44 species sampled for the experiment, 36 species were also found among all samples of the monitoring program that have already been identified (not only referring to the 81 samples). Eight species were unique in the

Table 3
Number of individuals (ind.), biomass (mg) and respective proportions (%) of the whole sampling (425 ind., 6987.5 mg) of the four most frequent spider families sampled for the experiment. In comparison, the corresponding data on the number of individuals per family from the monitoring samples.

	Experiment				Monitoring	
Family	ind.	%	mg	%	ind.	%
Lycosidae	230	54	5370	77	954	69
Thomisidae	16	4	381	6	54	4
Gnaphosidae	13	3	366	5	20	1
Linyphiidae	111	26	282.5	4.0	220	16
Remainder	55	13	588	8	140	10

experimental samples. The largest pseudosample of the experiment comprised 43 spiders with a total fresh mass of 757 mg (± 1.115 mg). The smallest pseudosample included five spiders with a fresh mass of 77 mg (± 0.380 mg); the mean was 21 spiders per sample (Supplementary Table S3). From the 81 monitoring samples, the largest sample comprised 72 spiders, the smallest sample one spider; the mean was 17 spiders per sample.

3.2. Comparison of biomass estimation methods

Both the absolute and relative deviation from fresh mass differed among methods (Fig. 2, Table 4). Since the absolute deviation of the estimates was positively correlated with the biomass for most, but not all regression models, we used the relative deviations to compare the different models (statistical data for correlations between fresh mass and absolute deviation are reported in Supplementary Table S5).

The lowest mean relative deviation from fresh mass (0.9%) resulted from the regression model based on ground spiders using the species-specific mean body lengths from a public database ($BL_{gr,\ mean}$), followed by the regression model based on all spiders (BL_{mean}: −1.5%). Both estimates (Fig. 2, Table 4) were not significantly different from the fresh mass, but came with a high variability (21.6% & 19.5%). The

Table 4
Mean relative deviations (rel. dev.) of the different regression models and drained weight from fresh mass across the 20 pseudosamples, with corresponding 95% confidence intervals (CI), *p*-values for the test against zero deviation and standard deviation (SD). DW = drained weight, FM_{lyco} = fresh mass of only lycosids, BL = body length, PL = prosoma length, PW = prosoma width, mean = species-specific mean body length from a public database, gr = only ground spiders used for the regression.

Method	Mean rel. dev. [%]	95% CI [%]	*p*-value	SD [%]
DW	48.0	38.1 to 57.9	< 0.001	21.0
FM_{lyco}	−26.8	−37.7 to −15.9	< 0.001	20.8
BL_{mean}	−1.5	−11.4 to 8.5	0.764	19.5
$BL_{gr,\ mean}$	0.9	−10.0 to 11.8	0.867	21.6
BL	−24.8	−29.5 to −23.9	< 0.001	8.5
BL_{gr}	−22.3	−27.1 to −20.2	< 0.001	9.3
BL × PW	−13.7	−17.2 to −10.3	< 0.001	7.5
BL × PW_{gr}	−17.2	−20.7 to −13.6	< 0.001	7.5
PL	−9.8	−14.0 to −5.7	< 0.001	10.3
PW	1.9	−3.0 to 6.8	0.418	11.8

regression models based on body size measurements showed considerably lower variability (7.5–11.8%). All these models showed mean relative deviations from the fresh mass significantly different from zero ($p < 0.001$), with confidence intervals not including zero. The only exception was PW ($p = 0.418$). The regression models generally underestimated fresh mass, with mean relative deviations ranging from −24.8% for BL to 1.9% for PW. Drained weight strongly overestimated the biomass (+48.0%) along with a high variability (21%) (Table 4). The fresh mass of only lycosids (FM_{lyco}) also differed significantly from fresh mass, with a mean relative deviation of −26.8% ($p < 0.001$; confidence intervals not including zero).

The linear mixed-effects model revealed statistically significant differences ($p < 0.001$) between the methods. Pairwise post-hoc comparisons revealed that drained weight (DW) differed significantly from all regression models and consistently yielded higher mean relative deviation (Fig. 2). Pairwise comparisons between the models using measured body length and using species-specific mean body lengths also showed

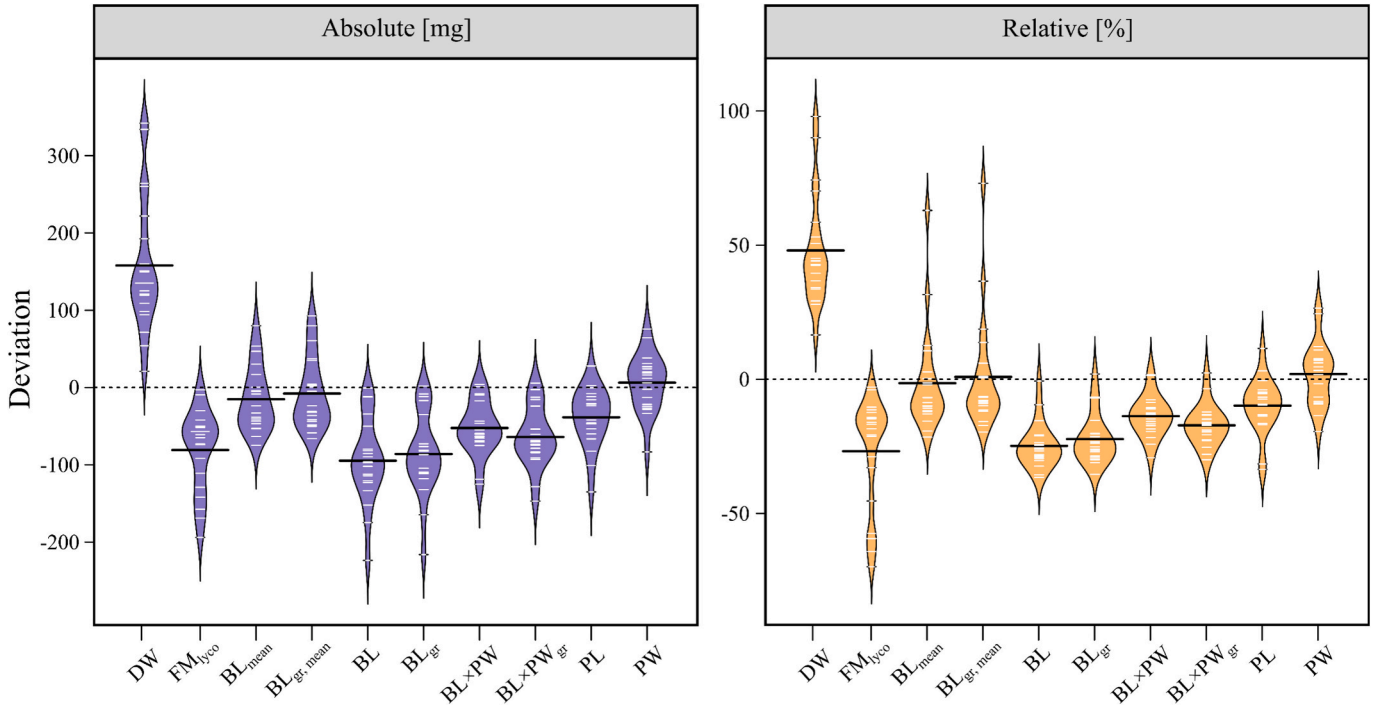


Fig. 2. Beanplots showing the distributions of absolute and mean relative deviations of the different regression models and drained weight from fresh mass across the 20 pseudosamples. The black horizontal lines show the mean deviation for each group. DW: drained weight, BL = body length, PL = prosoma length, PW = prosoma width, mean = species-specific mean body length from a public database, gr = habitat specificity for ground spiders.

significant differences (BL - BL_{mean}: $p < 0.001$, BL_{gr} - $BL_{gr,\ mean}$: $p < 0.001$) (relative estimates, *t*-ratio and *p*-values for pairwise comparisons are reported in Supplementary Table S4).

Comparisons of drained weights from the 81 monitoring samples with biomass estimates derived from the regression models consistently revealed strong and statistically significant ($p < 0.001$) relative deviations (Fig. 3, Table 5). Drained weight was higher than estimated biomass in nearly all samples. The highest mean relative deviation was found for BL_{mean} (−59%), whereas the lowest was found for BL_{exp} (−39.5%). Biomass calculated using the own regression model based on the sampling for the experiment (BL_{exp}) was higher (deviation from DW lower), with slightly higher standard deviations (drained weights and biomass estimates per regression model for all 81 monitoring samples are reported in Supplementary Table S6).

Table 5
Mean relative deviation (rel. dev.) of the different regression models from the drained weight across the 81 monitoring samples with *p*-values for the test against zero deviation and standard deviation (SD). BL = body length, PL = prosoma length, PW = prosoma width, mean = species-specific mean body length from a public database, gr = only ground spiders used for the regression, exp. = regression models based on data from the experiment.

Method	Mean rel. dev. [%]	*p*-value	SD [%]
BL_{mean}	−59.0	< 0.001	16.0
$BL_{gr,\ mean}$	−58.7	< 0.001	16.7
BL	−56.5	< 0.001	14.1
BL_{gr}	−56.0	< 0.001	14.4
BL × PW	−54.1	< 0.001	14.2
BL × PW_{gr}	−56.3	< 0.001	13.5
PL	−53.0	< 0.001	15.2
PW	−50.0	< 0.001	15.4
BL_{exp}	−39.5	< 0.001	20.0
$BL_{exp,\ mean}$	−43.2	< 0.001	23.5

4. Discussion

In this study, we used different regression models and compared them to drained weight, a method commonly employed in monitoring programs. We found substantial differences in accuracy and variability, with implications for the use of these methods in standard protocols. Based on our results we were able to find support for our hypotheses (1) and (2). Hypothesis (3) was partly supported and hypothesis (4) was not supported under given conditions in our experiment.

4.1. Performance of biomass estimation methods

Measuring biomass of spider samples by drained weight had the lowest accuracy, consistently overestimating the biomass of the samples. Even more important when discussing this in the context of a monitoring program, the variability of this method was higher than the variability of most regression models. Notably, the SD of drained weight (21%) was approximately three times higher than those of the regression models (7.5% for BL × PW, Table 4). This supports our hypothesis H1, that measuring drained weight is not the most accurate method to determine biomass of spider samples in monitoring programs and is associated with high methodological variability. All biomass estimates, based on body size-biomass regression models from the literature (Penell et al., 2018) - showed higher consistency with the actual fresh mass than the drained weight, generally underestimating the biomass of samples. All estimates based on individual body size measurements performed much better in terms of accuracy and variability and were therefore in line with our hypothesis H2. Hypothesis H3 was not supported by the results, at first sight. While the deviation compared to fresh mass of the regression model using species-specific mean body length of spiders from a public database is low and thus accuracy was high, its variability was also higher than that of models based on individual body size measurements. That higher variability was mainly attributed to two distinct outliers (pseudosample_06 and pseudosample_15). Looking at the raw data for all 425 spiders of the biomass experiment revealed that the largest outlier resulted from one single female specimen of the species *Drassodes lapidosus*. It showed an unusually small body length (8.98 mm) compared to the mean body length given for the species in the public database (11.5 mm; ARAMOB, 2026). This resulted in a considerable overestimation of the biomass of this specimen compared to the actual fresh mass. This bias was reflected clearly in the overestimation of the biomass of the whole sample (pseudosample_15), which consisted of only seven spider specimens. Removing the outliers decreased the variability of the two estimates considerably. For $BL_{gr,\ mean}$ the SD decreased from 21.6% to 10.2% and for BL_{mean} from 19.5% to 9.2%, well comparable with the SD of the measurement-based estimates. Simultaneously the mean deviation also increased from 0.9% to −5.1%

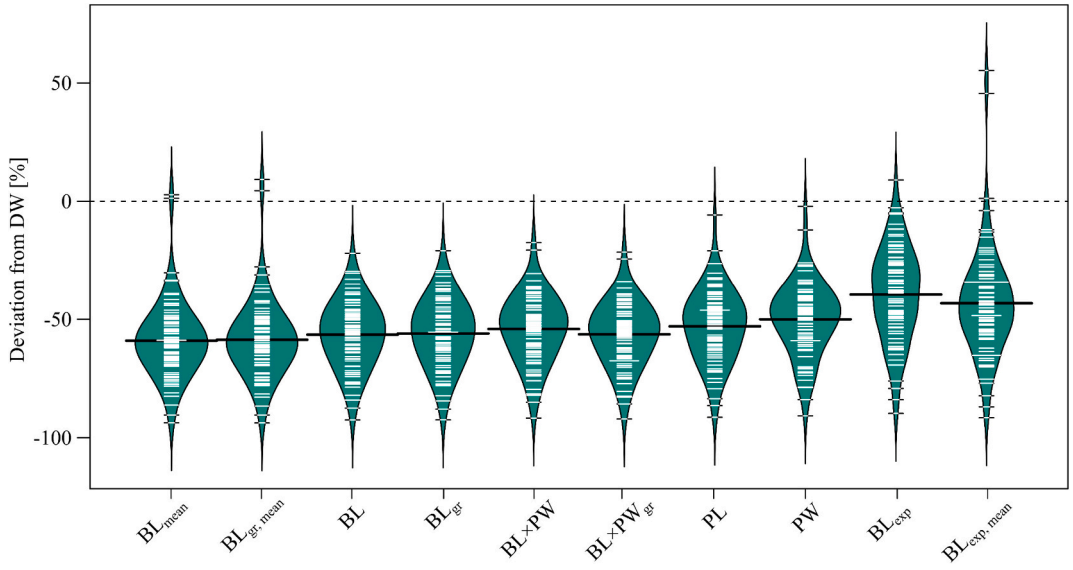


Fig. 3. Beanplots showing the distributions of mean relative deviations of the different regression models from drained weight across the 81 monitoring samples. The black horizontal lines show the mean deviation for each group. DW: drained weight, BL = body length, PL = prosoma length, PW = prosoma width, mean = *species-specific mean body length from a public database, gr = habitat specificity for ground spiders.* Note that deviations from DW (i.e. deviations from zero) should not be interpreted as greater accuracy, as unbiased fresh mass was not available for the monitoring samples.

for $BL_{gr,\ mean}$ and from −1.5% to −6.9% for BL_{mean} (Supplementary Table S7). The removal of outliers from the dataset is not a feasible method. It highlights that biomass estimates based on regression models can be highly sensitive to deviation of single individuals from a mean body length found in a public database or in literature, especially when the sample is small (i.e. few individuals; see also Weiss and Linde, 2022). Based on our knowledge and experience it should also be considered, that not all spider species are equally variable in size and that body size values stated in databases can vary on the respective species.

4.2. Influence of trapping and preservation fluids on biomass estimates

The general difference between biomass estimated by the regression models and biomass measured as drained weight observed in our experiment was also observed in the 81 real monitoring samples, with the same general direction of deviation. This suggests that we sampled a spectrum of epigeic spiders that was broadly comparable to that represented in the monitoring samples. Nevertheless, the differences between drained weight and biomass estimates were consistently lower for the monitoring samples (39.5%–59%) than expected based on the experiment data (46.1%–72.8%) except for BL_{mean}, $BL_{gr,\ mean}$ and PW. On the one hand, this may reflect differences in the source material from which the samples were drawn between the monitoring and experimental datasets, such as the number of samples and a higher number of specimens in some of the monitoring samples. On the other hand, it may also indicate an effect of the different trapping fluids used, namely propylene glycol (PG) in the experimental samples and Renner solution in the monitoring samples. Renner solution was deliberately not used in this experiment and PG was chosen to ensure the possibility of subsequent DNA analyses and because the spiders used for the development of the regression parameters by Penell et al. (2018) were also stored in PG. This limitation should be taken into account when directly comparing the regression estimates and monitoring samples. However, this limitation does not apply to the experiment results, since PG was consistently used as the trapping liquid. The relatively high overestimation of the drained weight is very likely a result of the penetration of fluid inside and adhesion on the body of the specimens. Also the density of the fluid may have an effect. Several studies have investigated the effects of preservation on the biomass of aquatic invertebrates (e.g. Dekanová et al., 2023; Wetzel et al., 2005). However, we are not aware of studies on spiders that examine the use of propylene glycol (PG) as a trapping fluid in combination with ethanol as a preservative, limiting the comparability of our findings. Nevertheless, evidence from other terrestrial arthropods suggests that trapping fluids can substantially affect biomass estimates. For example, Knapp (2012) reported a significant increase in wet biomass of carabid samples after 1 month of storage in propylene glycol under comparable conditions. Similarly, Braun et al. (2009) found significant increases in wet biomass of firebug (*Pyrrhocoris apterus*) samples after exposure to an ethylene glycol-based trapping fluid for 2 and 4 weeks. This effect could be even stronger for spiders as they are in general hairier than carabids and firebugs and their opisthosoma is also less sclerotized, which might lead to increased fluid penetration inside the specimen's body. The general underestimation of biomass by the regression models for BL, BL_{gr}, BL × PW, BL × PW_{gr} and PL could also be related to an influence of the trapping fluid on the morphological condition of the specimens after storage. This may be particularly relevant for body measurements that include the opisthosoma (i.e. measurements including body length, BL). For some spiders from the biomass experiment, the opisthosoma was strongly contracted after the storage in PG, possibly due to its hygroscopic properties. This could have led to reduced body lengths measured and thus to lower biomass estimates. Therefore, the results from the experiment apply to the propylene glycol and ethanol storage regime used in our experiment and may not be directly transferable to specimens stored in other trapping or preservation fluids. It also may explain the high mean deviation of body length-based models (BL, BL_{gr}) and the comparatively lower mean deviations observed for prosoma (PL, PW), combined (BL × PW, BL × PW_{gr}) and species-specific mean-based (BL_{mean}, $BL_{gr,\ mean}$) models.

4.3. Implications for selecting biomass estimation methods

Method selection should consider both the primary objective of the study and the level of accuracy and variability required to adequately detect the biological patterns or differences of interest. It should also consider the methodological effort that can realistically be invested in obtaining the biomass estimate (Fig. 4). When assessing spatial or temporal differences or changes in arthropod populations (e.g. monitoring programs), the variability of a biomass estimation method is more important than its absolute accuracy, as low variability is essential for reliably detecting population changes among sites or over time. To be more precise, if the method's variability exceeds a critical effect size, observed differences may reflect methodological variation rather than actual population trends. Based on the results from our experiment, regression models using body length (BL) and the combination of body length and prosoma width (BL × PW) would therefore be favourable for estimating the biomass of spider samples in monitoring applications, as they showed the lowest variability. In addition to our experiment, other studies also found that estimating biomass with regression models based on prosoma measurements solely is less reliable compared to body length, but that combining body length and body width leads to overall improvements (Gruner, 2003; Penell et al., 2018; Sohlström et al., 2018). Practically, this implies increased measurement effort resulting from the inclusion of an additional body dimension. This makes regression methods particularly suitable for smaller samples, although deep learning-based methods for automated arthropod body length and width measurement have already been developed that may help to reduce this limitation in the future (Shirali et al., 2026).

Methodological effort is therefore an important additional consideration when evaluating the suitability of a biomass estimation method.

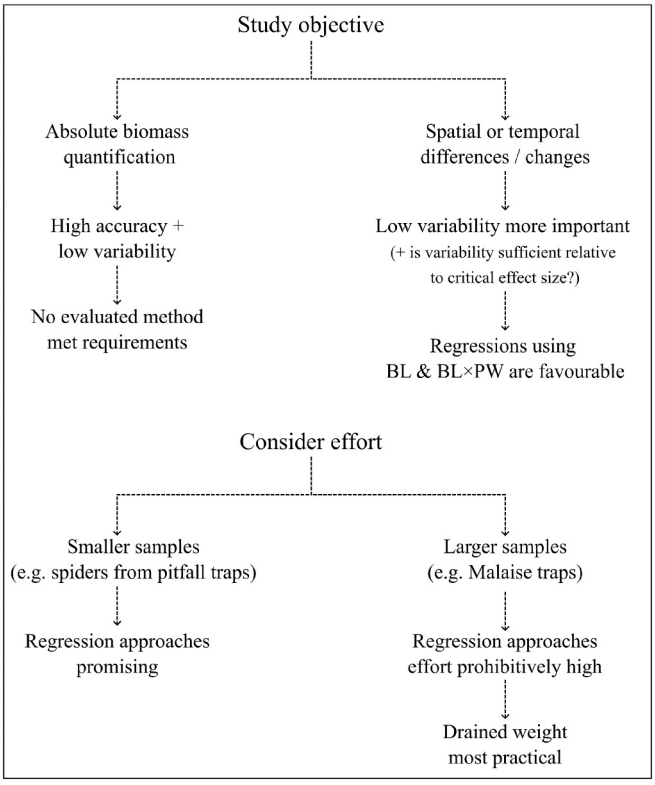


Fig. 4. Decision framework for selecting biomass estimation methods according to study objective, required accuracy and variability and methodological effort. BL = body length, PW = prosoma width.

A key advantage of the drained weight method lies in its practical applicability, as it can be implemented with relatively low time and effort, especially for large samples (e.g. malaise trap samples). In addition, the method is highly standardized and has been widely applied in studies investigating insect decline (e.g. Hallmann et al., 2017; Welti et al., 2021). However, this raises the question of whether its accuracy and variability can be improved without compromising these advantages. Köcher-Aust (2022) addressed this issue by testing an "optimized" drained-weighing method for Hymenoptera as an alternative to a standard approach similar to that used in our experiment. Her results show that the alternative approach significantly improved biomass estimation by having a lower variability. The conclusion was that the mass of ethanol evaporation and also the precision of the analytical balance play a crucial role in estimating biomass via drained weight, especially in very small samples. Another option would be to apply a correction factor to the drained weight. Additional analyses using linear regression models showed no clear relationship between the relative deviation of drained weight from fresh mass and sample size, the proportion of lycosids, or sample mean body length (Supplementary Table S8). Moreover, the ratio between fresh mass and drained weight was relatively consistent across the 20 pseudosamples (Supplementary Table S9). Together, these findings suggest that a correction factor could help compensate for the systematic bias in drained weight. However, a correction factor derived from this dataset should be used with caution outside the frame of this study. To develop a more universally applicable correction factor, further studies are needed to expand the dataset and thereby make it generally more robust for this approach.

If the aim of a study is to quantify the actual biomass of a spider community, for example to investigate food-web dynamics or nutrient cycling, methods with high accuracy and low variability are preferable. A key prerequisite for this is the use of regression models that are as specific as possible (taxon-specific, climate-region-specific, habitat-specific), as this substantially improves the accuracy of biomass estimates (Hódar, 1996; Höfer and Ott, 2009; Sohlström et al., 2018; Versluijs et al., 2023). This pattern was also evident in this study. In our experiment, however, none of the tested methods clearly met both requirements simultaneously. Either the methods exhibited significant mean deviations from fresh mass, or - when this was not the case (BL_{mean}, $BL_{gr,\ mean}$), their variability was high. For spiders in agricultural landscapes, nevertheless, biomass should always be analysed together with abundance to account for abundance changes and dominance shifts, which in turn can alter assemblage metrics like biomass (Wildermuth et al., 2025).

4.4. Limitations of biomass as a sole indicator of spider population change

A frequently recurring fact in monitoring programs is that due to the amount of work and a lack of financial resources or even taxonomic expertise, many taxa remain unidentified (Hochkirch et al., 2022; Wägele et al., 2022). That initially limits the application of certain methods like using species-specific mean body lengths for regression approaches. Wolf spiders (Lycosidae), which represent the largest size-class of epigeic spiders, exhibit the highest activity densities in pitfall traps (Edo et al., 2025; Kitt and Kielhorn, 2021) and on arable land (Plath et al., 2021), reflecting their active hunting mode. This raises the question of whether identifying them to species level and estimating their biomass would be sufficient to approximate the total spider biomass. In our experiment, lycosids made up 54% of the total specimens and 77% of total biomass followed by linyphiids (26% specimens; 4% biomass). Within the Epigeic Biomass monitoring program, spider samples collected over 3 years (>70,000 individuals) have already been taxonomically identified (Eberhardt et al., unpublished results). The same pattern was observed, with Lycosidae representing the most abundant family, accounting for 60% of all individuals, followed by Linyphiidae (26%). However, our results do not support the use of lycosid biomass as a reliable proxy for total sample biomass. Fresh mass based solely on lycosids (FM_{lyco}) systematically underestimated total fresh mass (FM) across all 20 pseudosamples, with a mean relative deviation of −26.8% (Table 4). As non-lycosid biomass was excluded, this systematic underestimation was expected, although not in this dimension. Using the same approach as for DW, we investigated whether applying a single correction factor to FM_{lyco} would allow reliable approximation of total sample fresh mass. Additional analysis using linear regression showed no clear relationship between the relative deviation of FM_{lyco} from fresh mass and number of non-lycosid individuals (Supplementary Table S8). However, the ratio between FM_{lyco} and FM varied considerably across the 20 pseudosamples (Supplementary Table S9). Thus, even under conditions where lycosids dominated both activity density and biomass, applying a single correction factor to their fresh mass would not reliably account for variation in total sample biomass, and restricting biomass determination to this dominant family therefore did not provide a sufficiently reliable estimate of total sample biomass. Biomass estimates of spiders obtained using activity-based sampling methods, such as pitfall traps, should therefore be interpreted with caution. Our experiment suggests that total biomass can be strongly influenced by large-bodied and mobile taxa like lycosids. Although this relationship was not statistically significant in our study, it again highlights the importance of considering biomass alongside activity density and species diversity rather than using biomass alone as a surrogate for the number of individuals. Biomass alone may obscure underlying changes in spider assemblages because changes in the abundance of species with different body sizes can compensate for each other at the level of total biomass. For example, an increase in one large-bodied lycosid species could mask a decline in numerous smaller-bodied linyphiid species. Considering species composition and activity density alongside biomass would also allow the use of species-specific mean body length regression models. This is particularly relevant given recent evidence for declines in small web-building spiders, including linyphiids, in arable land and more generally (Samu et al., 2023; Muster et al., unpublished results).

5. Conclusion

Our study evaluated the accuracy of different biomass estimation methods with respect to actual fresh mass of epigeic spider samples collected in anthropogenically influenced habitats in Germany, while also assessing their methodological variability. We discuss potential causes of the observed patterns and the consequences. Method selection should consider the study objective, the required accuracy and variability, and the methodological effort needed to obtain biomass estimates. At present, regression models appear promising for estimating biomass in smaller samples, such as spiders collected from pitfall traps in monitoring programs. Their lower variability compared to drained weight supports their use for such applications. These models can use individual body measurements (e.g. body length) when specimens are not identified further, or species-specific mean body lengths from public databases when identification is available (while accounting for sensitivity to outliers). In our experiment, PG was deliberately used instead of Renner solution to allow subsequent DNA analyses and because the spiders used to develop the regression models by Penell et al. (2018) were also stored in PG, limiting the direct comparison between the regression estimates from our experiment and the monitoring samples. However, this limitation does not apply to comparisons among methods within our experiment. Estimating biomass of large taxa only (like lycosids in our study) seems not to be a feasible approach, even if the taxon is clearly dominant in the investigated habitat and is effectively captured by the sampling method used (for lycosids, activity-based methods). For larger samples, such as malaise trap samples comprising thousands of arthropods, the effort required for regression approaches appears to be prohibitively high. In such cases, using the drained weight remains the most practical and likely the only feasible approach for estimating biomass and ensuring comparability across spatial and

temporal scales. Nevertheless, the results of our study, demonstrating that regression approaches provide precise biomass estimates for spiders in ecological samples. They represent an important step towards the development of future AI-based automated processes for handling ecological samples and therefore to overcome one major challenge of long-term monitoring programs.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used ChatGPT in order to improve the readability and language of the manuscript. It was also used to help create the R scripts. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Julian Eberhardt: Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hubert Höfer:** Methodology, Investigation, Conceptualization. **Andreas Toschki:** Resources. **Viktoria A.W. Garau Valens:** Investigation. **Martin Husemann:** Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoinf.2026.104120.

Data availability

The physical spider specimens collected for the experiment and monitoring study are deposited as vouchers at the Natural History Museum Karlsruhe. All data, metadata and R scripts required to reproduce the analyses presented in this study are publicly available through Zenodo (Doi: https://doi.org/10.5281/zenodo.23593339). In addition, all measured traits, including morphological measurements and body mass values, are publicly available through the World Spider Trait database (Doi: https://doi.org/10.57758/8N2R-HD40).

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