# EFFECTS OF LAND-USE INTENSIFICATION AND ENVIRONMENTAL STRESSORS ON INTRASPECIFIC FUNCTIONAL TRAITS VARIABILITY IN BUMBLEBEES

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## ABSTRACT

1. Landscape anthropization replaces natural areas with either agricultural or urban covers, leading to land-use intensification. This worldwide phenomenon affects biodiversity, but little is known about the effects on the intraspecific variation of functional traits in spite of their importance for ecosystem functioning and services.
2. Here we investigated if the functional traits of pollinator insects responded to land-use intensification and environmental stressors, by characterizing the variation of important traits for flight performance that are also indicators of stressors during organism development. The possible impact of different land cover (i.e. semi-natural, urban and agricultural) and of multiple biotic and abiotic stressors (i.e. temperature, resource availability and air pollutants) was investigated.
3. Along a gradient of landscape anthropization, we quantified the impact of biotic and abiotic factors on two European bumblebee species (*Bombus terrestris* and *B. pascuorum*) sampled at 37 sites in Northern Italy. Through geometric morphometrics we investigated the variation of multiple morphological traits, namely wing centroid size (as a proxy of body size) and asymmetry of wing shape and size. Data on land-cover, climatic conditions and air pollutants were retrieved through remote sensing, while the estimation of resource availability was conducted directly in the field.
4. Our results highlighted the relevance of the two main land-use alteration trajectories (i.e. urbanization and agriculture intensification), although the two

species responded idiosyncratically. Urbanization led to shifts towards smaller body sizes due to increased impervious cover and air temperature in *B. pascuorum*. Instead, *B. terrestris* responded to agriculture intensification with larger body size in response to higher farmland cover and floral resource availability. Asymmetry in wing shape was not affected by abiotic factors of land-use intensification, but wing size asymmetry was positively associated with warmer temperatures and increased level of $NO_2$ in *B. terrestris*.

5. This study describes patterns of how landscape anthropization shapes pollinator insects' functional traits: urbanization is associated with smaller bees, while agriculture intensification leads to larger bees. In addition, environmental stressors impacted wing size asymmetries and could compromise flight performance, particularly in warmer, more polluted habitats. This study indicates that the land-use intensification of anthropized landscapes determines functional trait variation at the intraspecific level and that these responses are highly taxon-specific even in syntopic related organisms.

**Keywords:** *Bombus*, functional diversity, functional ecology, geometric morphometrics, heat islands, phenotypic plasticity, pollination.

## INTRODUCTION

Widespread phenomena, such as the progressive expansion of agricultural and urban lands are driving deep changes on ecosystems, often by driving intraspecific phenotypic variation (Alberti et al., 2017; Nooten & Rehan, 2020). Plants and animals respond to these marked environmental variations by shifting their distribution (Colla et al., 2012) and phenology (Huchler et al., 2020), and/or shaping some morphological traits considered "functional", i.e. relevant for their ecology, fitness and behaviour (Alberti et al., 2017; Eggenberger et al., 2019). Trait variation due to environmental alteration is particularly relevant in those organisms responsible for ecosystem services, such as pollinators: changes in their traits could jeopardize the service provided (Buchholz & Egerer, 2020). For instance, land-use intensification and degradation (i.e. the growing proportion of anthropized or productive surfaces across landscapes) could lead to limited floral resource availability to pollinators (Steffan-Dewenter et al., 2001). This scenario, in turn, could impose changes in pollinator functional traits such as body size decline due to low food supplied to larvae. Furthermore, anthropized landscapes change local microclimates, with great influences on bees ecology, development and activities (Radmacher & Strohm, 2010). In this context, higher temperatures in cities due to the "heat island effect", could determine a shift towards smaller body size as an adaptation to reduce risk of overheating (Peters et al., 2016; Gérard et al., 2018a). Considering that the steady growth of the human population is driving a dramatic increase in the area allocated to intensive agriculture and urban expansion worldwide, new insights in the study of

pollinator response are necessary to predict which scenarios would likely occur for urban biodiversity and its mediated services.

Previous studies investigated the morphological responses of pollinators to anthropogenic pressures, mainly focusing on key functional traits, such as body size (e.g. Chown & Gaston, 2010; Eggenberger et al., 2019; Theodorou et al., 2020). In bees, this character responds rapidly to environmental changes (Chown & Gaston, 2010), it shows little heritability and its variation mainly depends on the amount of food received during the larval development (Couvillon et al., 2010). Bee size directly influences their mobility and so their foraging range (Greenleaf et al., 2007), but also determines the metabolism rate and resource needs of adult imagos, with larger bees having higher metabolism rate (Kelemen et al., 2019) and thus potentially being more susceptible to variation in floral resource availability leading to starvation (Couvillon & Dornhaus, 2010). However, to date, the investigation of pollinator body size variation in anthropogenic habitats yielded heterogeneous results. A recent study on bumblebees found bigger workers in cities (Theodorou et al., 2020). This pattern has been interpreted as an adaptation to longer flights for collecting resources, due to severe habitat fragmentation and dispersion caused by land use intensification (Greenleaf et al., 2007). Conversely, a study by Eggenberger et al. (2019) found smaller bumblebee foragers in cities, interpreted as an effect of both limited local resource availability and warmer temperature in urban areas.

A promising research field in the study of morphological traits is to identify possible stress biomarkers of habitat quality and population health status (Adams et al., 2001). The advantage of using trait variation to measure stress resides in the fact

that changes of phenotypes are detectable before a decrease in population viability occurs (Hoffmann et al., 2005). This is why quantifying traits variation could become an essential practice when evaluating local- and landscape-level stressors. A metric that has grown in popularity is the fluctuating asymmetry (FA) (Klingenberg, 2001; Beasley et al., 2013; Alves-Silva et al., 2018), defined as the presence of small, randomly placed deviations from perfect bilateral symmetry due to the occurrence of developmental instability, driven by exogenous environmental conditions (Klingenberg, 2015). FA differs from another type of bilateral asymmetry, the directional asymmetry (DA), that occurs when one specific side tends to be steadily larger than the other. While DA have a genetic basis and therefore could be less impacted by the environment (Palmer & Strobeck, 2003), the FA is considered a valid proxy of stress exposure to conditions that typically occur in anthropized environments (e.g. temperature, air pollutants, and pesticides) (Beasley et al., 2013). For instance, laboratory-based studies have demonstrated that $CO_2$ or low temperature leads to wing FA, supporting the possible role of traffic pollutant in determining developmental instability (Klingenberg et al., 2001; Hoffmann et al., 2002). Interesting, however, these studies also indicated how wing shape and wing size asymmetries had different responses to the same stressor type. This aspect is ecologically very relevant. Both wing size and shape are important functional traits in pollinators. This is because wing size is believed to be related to flight length and it influences metabolic costs (Fernandez et al., 2017; Soule et al., 2020), while shape is considered as important for flight maneuverability (Kolliker et al., 2003, Grilli et al.,

2017). Thus, their asymmetry could reduce flight performance ability and thus could reduce fitness and even impact on species interactions and pollination efficiency.

In order to characterize the effects of landscape anthropization and related pressures on pollinator insects, we quantified the morphological variation in two species of bumblebee (i.e. *Bombus pascuorum* and *B. terrestris*), by investigating different populations across a gradient of growing land-use alteration, from semi-natural areas to rural sites and urban places. Specifically, we chose the region surrounding Milan (Northern Italy). This is one of the major European cities, and lies on a territory that experienced a strong anthropogenic footprint and ongoing efforts are trying to mitigate it. We expected to find quantitative variation in bumblebee functional traits of body size and wing asymmetry in response to differently anthropized landscapes. Our hypothesis was that in anthropized areas, pressures eliciting a decrease in body size (e.g. high temperature and low floral resource availability) would be prevailing and would lead to smaller foragers in urbanized and agricultural habitats. We also expected to find increased wing size and shape FA in response to higher levels of biotic and abiotic stressors (e.g. resources abundance, temperature and air pollutants). Hence, our ultimate goal was to determine which type of response prevails and the putative pressures that are determining it to better understand the impact of landscape anthropization on the ecology of pollinator insects.

## MATERIALS AND METHODS

### Study species

This study was focused on two co-occurring species of bumblebee: *Bombus terrestris* (Linnaeus 1758) and *B. pascuorum* (Scopoli 1763). Both species are efficient pollinators, common in Europe, and can be found in different habitats from the natural to the agricultural and urban ones (Polce et al., 2018). Given these characteristics, these species are reliable models to investigate responses by pollinating insects to landscape anthropization (Eggenberger et al., 2019; Theodorou et al., 2020). The two selected species have slightly different foraging ranges, with estimated maximum dispersal range of 449 and 758 m for *B. pascuorum* and *B. terrestris*, respectively (Knight et al., 2005). Nesting habits are also dissimilar as *B. terrestris* builds its nest in subterranean holes, while *B. pascuorum* on top of or slightly beneath the soil surface (Goulson, 2010). Another important difference is represented by their dietary regimes with the *B. pascuorum* usually having a narrower trophic niche and a preference for deep-corolla flowers (Harder, 1985).

### Study design and sampling

Samplings were conducted at 37 sites (Fig. 1), mainly lying within the metropolitan area of Milan in July 2019, only on days with sunny and not windy weather conditions.

Study sites were randomly chosen using QGIS 3.10.11 in an area of about 1800 $km^2$. A minimum distance between sites of 1 km was imposed to avoid the non-independence of sites (Phillips et al., 2019) and is more than the mean foraging range observed for the two species (Knight et al., 2005). Study sites were distributed along a gradient of landscape anthropization, ranging from areas dominated by semi-natural hay meadows close to forest, to agriculture dominated landscapes (i.e. sites at the edges of crop fields of mainly large monoculture) or urban sites located in public parks included in the highly urbanized matrix of Milan. Five to six specimens for each species (i.e. foragers) were captured at each site using an entomological net. After collection, the insects were stored at -80 °C until further analysis.

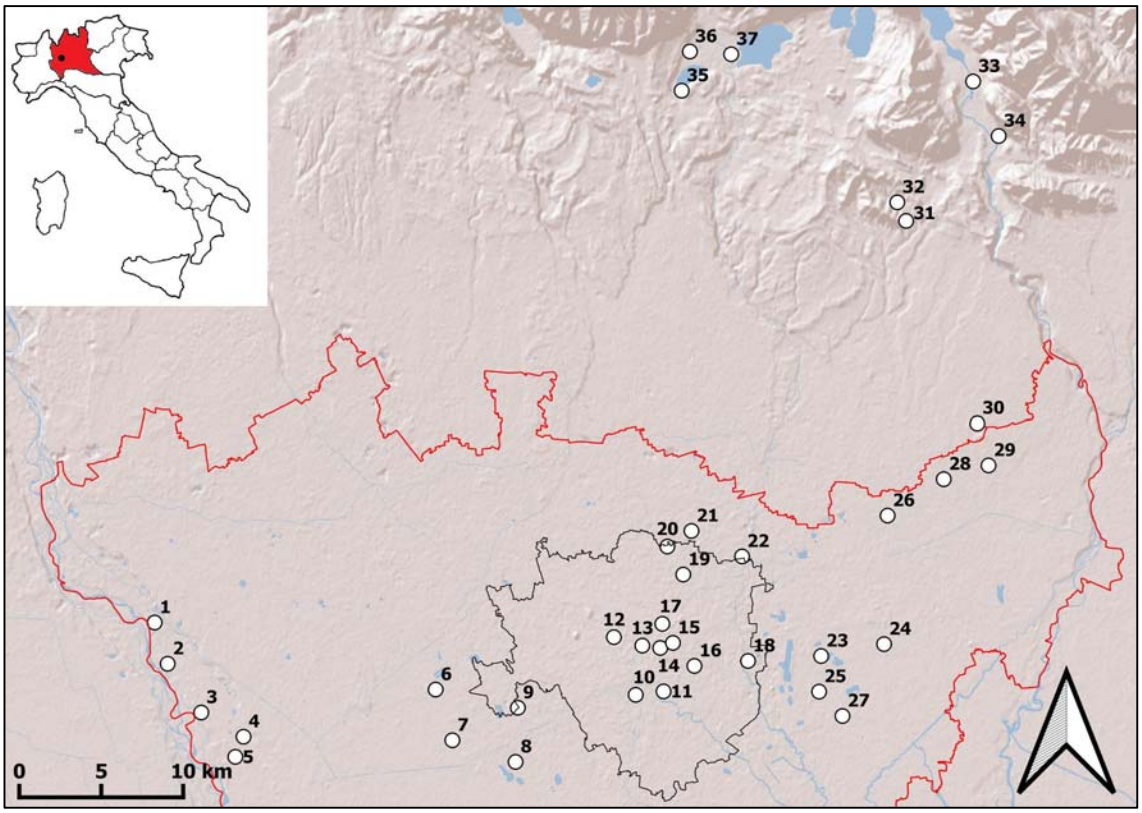


Fig.1: Map showing the locations of the sampling sites. The red line indicates the edges of the metropolitan area of Milan, the black line indicates the edges of the city of Milan.

## Landscape and environmental variables

In order to assess landscape anthropization, land-use data were obtained from the latest version of land cover database provided by Lombardy region (2018-DUSAF 6.0; https://www.dati.lombardia.it/Territorio/DUSAF-6-0-Uso-del-suolo-2018/7rae198 fng6). Using QGIS 3.10.11, a 1 km radius buffer area was created around each site to estimate landscape and environmental features. Landscape composition was evaluated arranging DUSAF original level and sub-level of land-use classification into three macro categories: impervious (i.e. buildings, infrastructures, roads and cemented surface), agricultural (i.e. arable land devoted to crop production) and semi-natural land (i.e. meadows, forest and urban green spaces) (Appendix S1 for a list of DUSAF codes assigned to each grouping). For each site, the ratio between impervious and semi-natural land (Impervious / Natural) as well as the ratio between agricultural and semi-natural land (Agricultural / Natural) was computed to describe the urbanization and agricultural intensification processes, respectively. Habitat fragmentation was quantified by computing the edge density (ED), as the ratio of edge length of green and semi-natural patches over their total area (Wang et al., 2014).

Other biotic and abiotic features of the selected study sites were considered to test for their potential effects on altering body size and wing size/shape asymmetry. Land surface temperature was calculated as the mean value in the period June-July using data retrieved through remote sensing imaging spectroradiometer (MODIS) MOD11A2 from the NASA database (https://modis.gsfc.nasa.gov/data/dataprod/mod11.php) with a resolution of 1 km. The

choice of considering temperature for these two months was dictated from the supposition that our sampled specimens developed during that period, according to the biology of two selected species (Goulson, 2010). Air pollution was estimated as the mean of daily concentrations of $NO_2$ over two months (June and July) registered by Regional agency for environmental protection (ARPA) through monitoring stations located nearby our sampling sites (https://www.arpalombardia.it/Pages/Aria/qualita-aria.aspx).

Resource abundance (i.e. the total number of flowers) was estimated by using six quadrats 1 m x 1 m and summing the number of flowers found within each quadrat, in 31 out of 37 sites.

## Specimens imaging and wings measurement

The forewings of all individuals were detached at the base and scanned at high resolution. The obtained images were converted into TPS files using tps-UTIL 1.74 and digitised using the tps-Dig 2.31 software (Rohlf, 2015), with two-dimensional cartesian coordinates of 15 landmarks positioned at wing vein junction (Fig. 2) (as in Aytekin et al., 2007; Klingenberg et al., 2001). Bumblebees with damaged or over weared wings were excluded from further analyses.

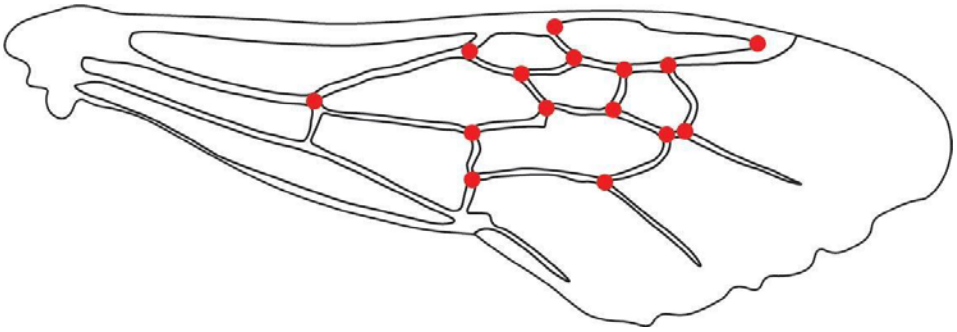

Fig.2: Right forewing of *B. terrestris* with landmark locations

The analysis of landmark configuration was conducted in MorphoJ 1.07 software (Klingenberg, 2011). In order to remove all differences unrelated to shape (i.e. rotation, translation and scale) a generalized least square Procrustes superimposition was applied (Klingenberg, 2011). The output of this procedure is a new set of superimposed landmark coordinates, called 'Procrustes shape coordinates', that contains all the shape information. Wing size was estimated as the centroid size (i.e. the square root of the sum of squared distances from the centroid of each landmark configuration) and used as a proxy of body size (hereafter "body size", as in Outomuro & Johansson, 2011 and Dellicour et al., 2017). Wing size asymmetry was computed by dividing the absolute difference between left and right centroid sizes by the mean centroid size and multiplying by 100 (Leonard et al., 2018). To estimate wing shape variation, Procrustes distances were computed for each individual (Klingenberg, 2015). These represent the measure of an individual's overall asymmetry (i.e sum of DA and FA components), obtained by taking the square root of

the sum of squared distances between corresponding right and left Procrustes' coordinates.

## Statistical analysis

Since the levels of asymmetry in bilateral traits are subtle, we estimated the measurement error, which could possibly cause considerable variation in the assessment of asymmetry levels, by double-scanning wings and digitizing their landmarks for a subset of 50 specimens, in MorphoJ with the Procrustes ANOVA (Klingenberg, 2001), in order to evaluate if measurement error was negligible. The presence of directional asymmetry (DA) was also tested with the same approach, and if DA occurred, the total asymmetry measures were corrected subtracting the mean DA from the overall individual asymmetry, as in Costa et al. (2015).

To investigate the relationship between morphological traits and landscape or environmental variables, linear mixed models were used. The response of the two species was assessed separately and collinearity among predictor variables was tested. Variables that showed heteroscedasticity of the residuals were log-transformed as reported in Table 1 and the goodness of fit of the transformations was assessed through AIC criterion. Response of centroid size was tested in two different models to avoid collinearity. The first one tested the hypothesis that foraging distances would change depending on landscape features; wing centroid size was included as response variable and edge density of green areas as well as the ratio between impervious or agricultural and semi-natural land-cover as predictor variables. The second model looked at investigating the possible impact on bumblebee size of different stressors,

with temperature and resource availability as predictor variables and again wing centroid size as response. Regarding asymmetry, we tested wing size and shape asymmetry in distinct models, with temperature, $NO_2$ and resource abundance as predictor variables. In all models the sampling site was included as the random effect. For all models we applied a stepwise model selection based on AIC values removing variables that did not improve the model fit (Zuur et al., 2009). Statistical analyses were conducted using MorphoJ 1.07 and JMP 14.2.0 (© SAS Institute Inc. Cary, North Carolina, US).

## RESULTS

Overall, the sampling dataset was similar in the two bumblebee species, encompassed a total of 179 *B. pascuorum* (mean per site = 4.8±0.4) and 169 *B. terrestris* (mean per site = 4.5±0.3) that were considered for morphometric analyses. The measurement error resulted as negligible because not significant for both wing size (df = 79, F = 2.67 p = 0.4578) and shape (df = 2054, F = 0.51, p = 0.9976). Both species showed a significant level of shape DA (*B. pascuorum* df = 26, F = 4.66; $p < 0.0001$; *B. terrestris* df = 26, F = 5.60; $p < 0.0001$), while size DA was statistically significant only in *B. pascuorum* (df = 1, F = 29.77; $p < 0.0001$; in *B. terrestris* df = 1, F = 0.51; p = 0.4779). Measures of FA were then obtained correcting for directional components.

Different patterns of size variation were found in the two bumblebee species. *B. pascuorum* decreased its body size in response to both the higher ratio of impervious

on semi-natural land cover (Fig. 3a; Table 1) and to increasing temperature (Fig. 3b; Table 1). Conversely, no significant effect of these two predictor variables was found on *B. terrestris*, that instead increased body size in response to agricultural cover (Fig. 3c; Table 1) and with increasing floral resource availability (Fig. 3d; Table 1).

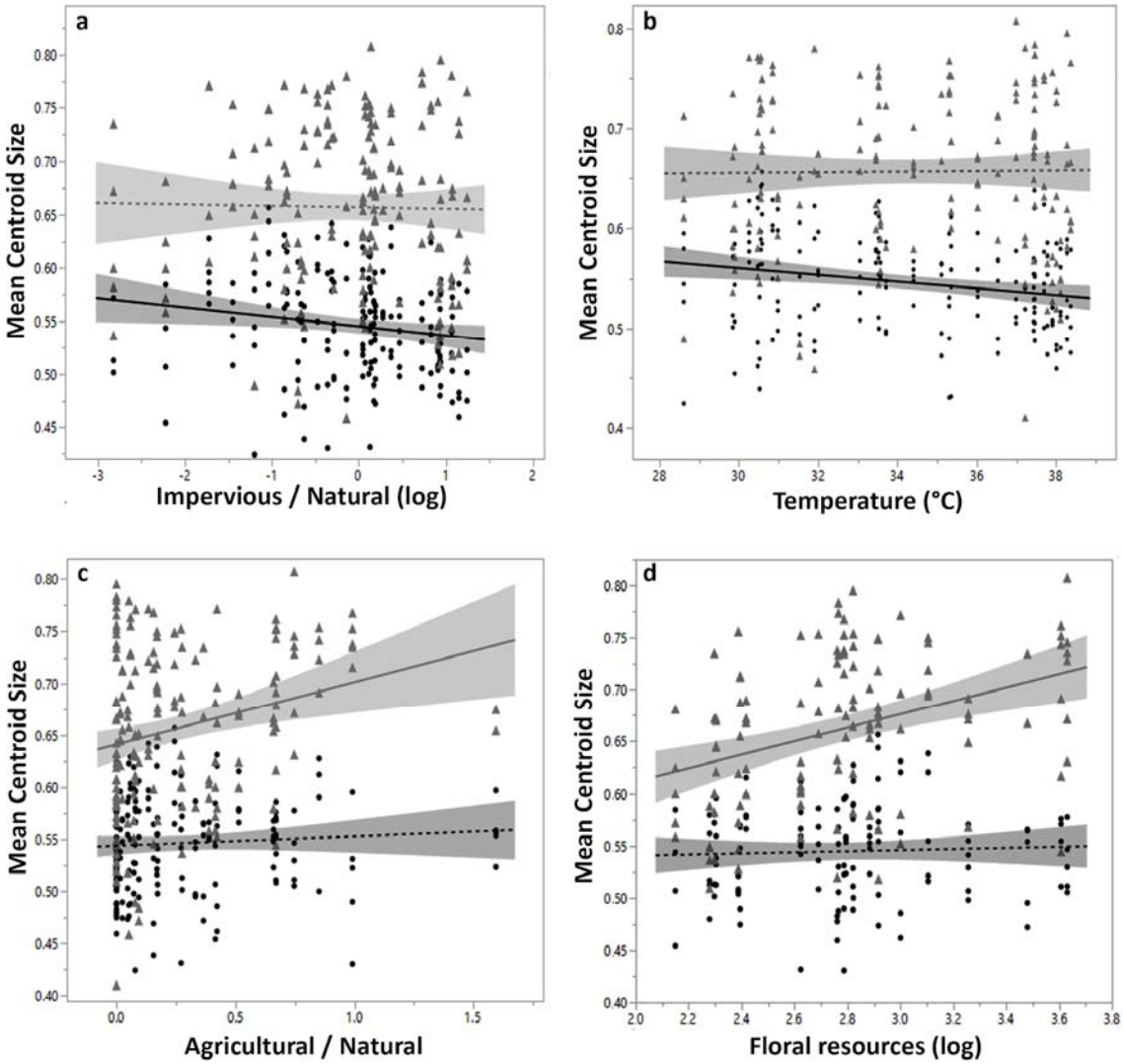


Fig. 3: Regression analysis of centroid size as a function of the amount of the ratio between impervious and semi-natural land (a), temperature (b), ratio between agricultural and semi-natural land (c), and floral resources (d).

*B. pascuorum* is represented by black dots and lines, *B. terrestris* is represented by grey triangles and lines. Continuous lines indicate the significant relationships while dashed lines represent the non-significant ones.

Dots and triangles represent observed values. The two species were tested in separate models but represented together to facilitate the comparison

Concerning wing size FA, *B. terrestris* showed high levels in response to increasing temperature and concentration of $NO_2$ (Fig. 4a and b, respectively; Table 1), but no such effects were found in *B. pascuorum.* None of the predictor variables (i.e. temperature, $NO_2$, resource abundance) showed any significant effect on wing shape asymmetry in both bumblebee species.

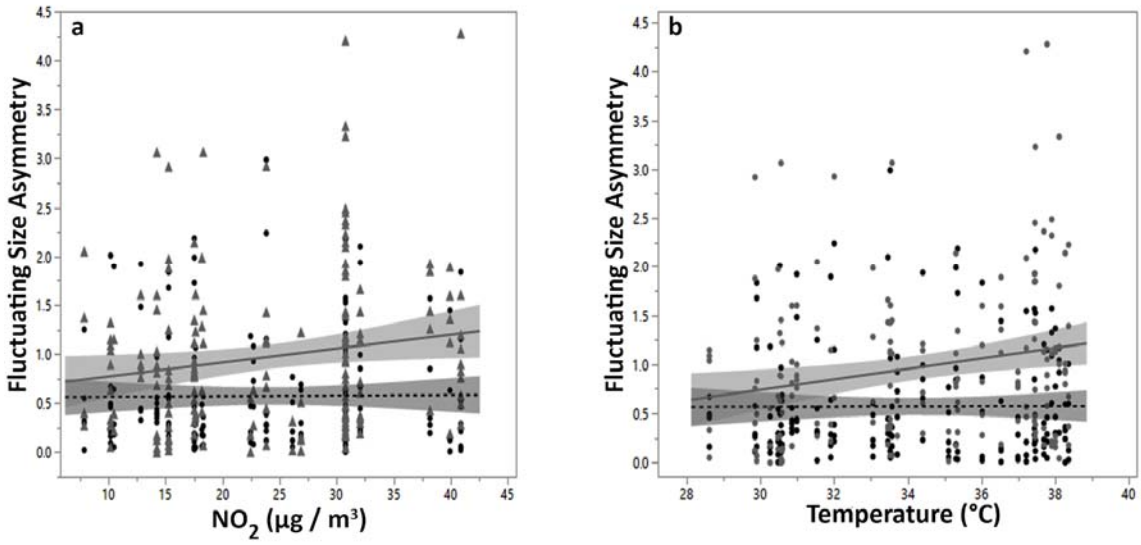


Fig. 4: Regression analysis of fluctuating size asymmetry as a function of the amount of N02 (a), and temperature (b). *B. pascuorum* is represented by black dots and lines, *B. terrestris* is represented by grey triangles and lines. Continuous lines indicate the significant relationships, while dashed lines represent the non-significant ones.

| Response variable | Model | Predictors | Bi | Df; F | P > F |
|---|---|---|---|---|---|
| *B. pascuorum* Centroid size | (1) Potential foraging dispersal | Impervious/Natural (Log) | -0.00869 | 1; 4.2661 | **0.0462** |
| | | | | | |
| | (2) Environmental stressors | Temperature | -0.00341 | 1; 7.7271 | **0.0088** |
| *B. terrestris* Centroid size | (1) Potential foraging dispersal | Agricultural/Natural | 0.06091 | 1; 5.7683 | **0.0211** |
| | | Edge density | -0.30599 | 1; 0.5124 | 0.4791 |
| | | | | | |
| | (2) Environmental stressors | Floral resources (Log) | 0.06560 | 1; 9.5747 | **0.0048** |
| *B. terrestris* Fluctuating size asymmetry | (1) Environmental stressors | Temperature | 0.05259 | 1; 11.5565 | **0.0019** |
| | | | | | |
| | (2) Environmental stressors | $NO_2$ | 0.01433 | 1; 7.2304 | **0.0118** |

Table 1**:** Results of the regression analysis of centroid size and fluctuating size asymmetry as a function of landscape and biotic and abiotic covariates. $\beta_i$: regression coefficient; F: F-value; P: p-values (significant value are in bold). Models and results of size FA for *B. pascuorum* and of shape FA for both species are not reported in the table as they were inconsistent.

## DISCUSSION

In this study, we quantified the spatial intraspecific variation of functional traits playing a crucial role for flight performance in two common bumblebee species. We focused on their variation along the main trajectories of land use alteration, namely urbanization and agriculture intensification of landscapes, by relating trait variation and wing asymmetries with landscape features and environmental stressors. This investigation sheds light on patterns of trait variation, to better describe how landscape anthropization shapes pollinating insects.

Landscape urbanization determined intraspecific variation of body size (i.e. estimated as wing centroid size) across bumblebees populations. Specifically, foragers of one of the two investigated species, *B. pascuorum*, showed a shift toward smaller body size in response to increasing levels of urbanization and also to higher urban temperatures. A similar pattern of body size reduction in urban bumblebees was reported by Eggenberger et al. (2019) and it agrees with observations showing a general decrease of body size in urban insect communities (Merckx et al., 2018). A possible driver of smaller sizes in urban bumblebees is the diminished, and spatially dispersed, availability of floral resources that is often associated with higher

impervious cover (Eggenberger et al., 2019). However, in our study, nor field estimates of floral abundance, nor green areas fragmentation (i.e. the edge density) correlated with size variation of *B. pascuorum*, the species that responded to urbanization. Hence, a reliable driver of urban-induces size shrinkage, as confirmed by the results of our study, could be warmer temperatures due to the urban "heat island effect" (Merckx et al., 2018). Indeed, temperature has a strong effect on insect body size, with multiple historical series collections-based and experimental studies that revealed how higher environmental temperatures represent a driver of body size reduction in different species of bees (e.g. Nooten & Rehan, 2020; Theodorou et al., 2020). Higher temperature impacts by accelerating larval development, which would result in smaller adults (Sibly & Atkinson, 1994). Furthermore, smaller sizes in warmer areas could also be a strategy for reducing overheating risks while foraging, due to an increased convective heat loss in smaller bees (de Farias-Silva & Freitas, 2020). Functionally, smaller foragers could travel shorter foraging distances (Greenleaf et al., 2007) and could also load less pollen and nectar (Goulson et al., 2002). As a consequence, the shift towards smaller body size in *B. pascuorum* in urban environments could imply that it will pollinate less plants or handle flowers less efficiently (Földesi et al., 2020), a concerning aspect in view of colony provision and pollination.

The intensification of agriculture landscapes was positively associated with *B. terrestris* body size. A possible explanation is that a larger body size can be required in agricultural areas where resources-rich patches are interspersed between large monoculture crops (Gonzalez-Varo et al., 2013; Hillaert et al., 2018) in order to

facilitate the movement among distant resource-rich patches. Similar patterns of response were observed in other studies on differently sized bee species. Gérard et al. (2020) reported larger bumblebee queens in agricultural landscapes. Similarly, Kelemen & Rehan (2020) observed larger females of the small carpenter bee *Ceratina calcarata* in response to increasing levels of agricultural land cover. Furthermore, *B. terrestris* in our study responded to resource availability, an important result showing that generalist pollinators need a high amount of resources (e.g. Biella et al., 2019). Hence, these results indicate that large bumblebees accessing higher flower amounts in open, agricultural landscapes would be, in turn, also better able to exploit and forage across agricultural areas.

Despite *B. pascuorum* and *B. terrestris* belong to the same genus, the responses of these two species were not equal. *Bombus terrestris* body size did not vary in urban environments, while *B. pascuorum* did not respond to agricultural landscapes. Similarly, idiosyncratic responses were also observed in other bumblebee species, where if the body size of some decreased over warming decades, others responded in the opposite way (Gérard et al., 2020). These species-level, idiosyncratic responses are very relevant for our understanding of the potential mechanism of intraspecific trait variation associated with landscape anthropization. This supports the need to consider a wide panel of species when investigating the impact of landscape anthropization on biodiversity. For instance, the invariant size of *B. terrestris* in warmer cities is likely due to the higher heat tolerance of this species compared to *B. pascuorum* (Martinet et al., 2020). In addition, *B. terrestris* nests further underground compared to the other bumblebee, and might be less exposed to warm, urban air

temperatures during larval stages. These aspects strengthen the hypothesis that temperature is a major determinant of pollinator size reduction in cities, because they affected the body size of the more temperature-sensitive species, but not the heat-tolerant one.

Flight performance does not only depend on body size, but it is also affected by asymmetries in shape and size between wings. Here, we detected high wing size FA (i.e. fluctuating asymmetry) in response to environmental stressors such as higher temperatures and $NO_2$ concentration in *B. terrestris*. The absence of variations in shape asymmetry registered for both the bumblebee species could confirm the results from other studies that have indicated shape variation as less susceptible to stressors than size asymmetry (e.g. Gérard et al., 2018b). The effect of temperature on wing asymmetry was observed in other insect taxa under controlled laboratory conditions (Mpho et al., 2002) and field exposure to road traffic (Leonard et al., 2018). Studies associated the increased wing FA to environmental stressors, indicating that impairment of developmental processes might take place (e.g. Klingenberg et al., 2001; Kerr et al., 2013). For the case of pollutants, Klingenberg et al. (2001) hypothesized that they could alter development through mechanisms related to gas exchange. Trait variation does not only show developmental instability, but it also has ecological implications. Wing size FA impacts the management of lengthy flights (Fernandez et al., 2017, Soule et al., 2020), compared to wing shape which is often associated with flight maneuverability. Thus, size asymmetry could specifically impact flight performance, flight length in time and space, and even affect bumblebee interactions with plants, the provisioning of larvae with food and their pollination rate.

## CONCLUSIONS

Overall, the experimental results obtained in this study indicate how landscape anthropization could affect different functional traits, and it does so in different pollinator species. Eventually, these responses could bring to the alarming outcome of decreased pollination efficiency, because the studied traits are often involved in flying abilities. Temperature emerged as one of the main drivers of these phenotypic variations, affecting the two species in a different manner. The multiple responses to the same stressor, observed for the two species, underline the necessity for future studies to consider a wider panel of taxa instead of single model species.

From a conservation perspective, the comprehension of how pollinators cope with the challenging conditions occurring in novel anthropogenic habitats, plays a key role in informing suitable policy efforts to conserve their biodiversity and the ecosystem service they provide. In the future, cities are predicted to expand constantly and thus designing of urban landscapes will become a fundamental step for achieving sustainability outcomes. The creation and the wise management of urban green spaces will allow not only to create conditions that could support pollinators species abundance and diversity but also sustain the biological interactions that are the basis of a correct ecosystem functioning. At the same time, urban forestry and greenery practices (e.g. plantation of street and residential trees and the creation of urban greenbelts or greenways) could represent a valid solution to mitigate stressful conditions related to the urban environment, such as the “heat island effect” (Chun &

Guldmann, 2018) and the high level of air pollution (Ottosen & Kumar, 2020) that were here demonstrated to directly affect functional diversity issues.

## DATA AVAILABILITY STATEMENT

All relevant data are within the paper or stored in a public repository (https://doi.org/10.6084/m9.figshare.13637594).

## COMPETING INTERESTS STATEMENT

The authors declare that no competing interests exist.

## ETHICS STATEMENT

Sampling permits were obtained when needed from local authorities

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## AUTHOR CONTRIBUTION

NT, EP and AG conceived the ideas and designed methodology; NT, EP and PB collected the data; NT and EP analysed the data, PB contributed to analyses checking/development and checked the final model outcomes; EP, NT and PB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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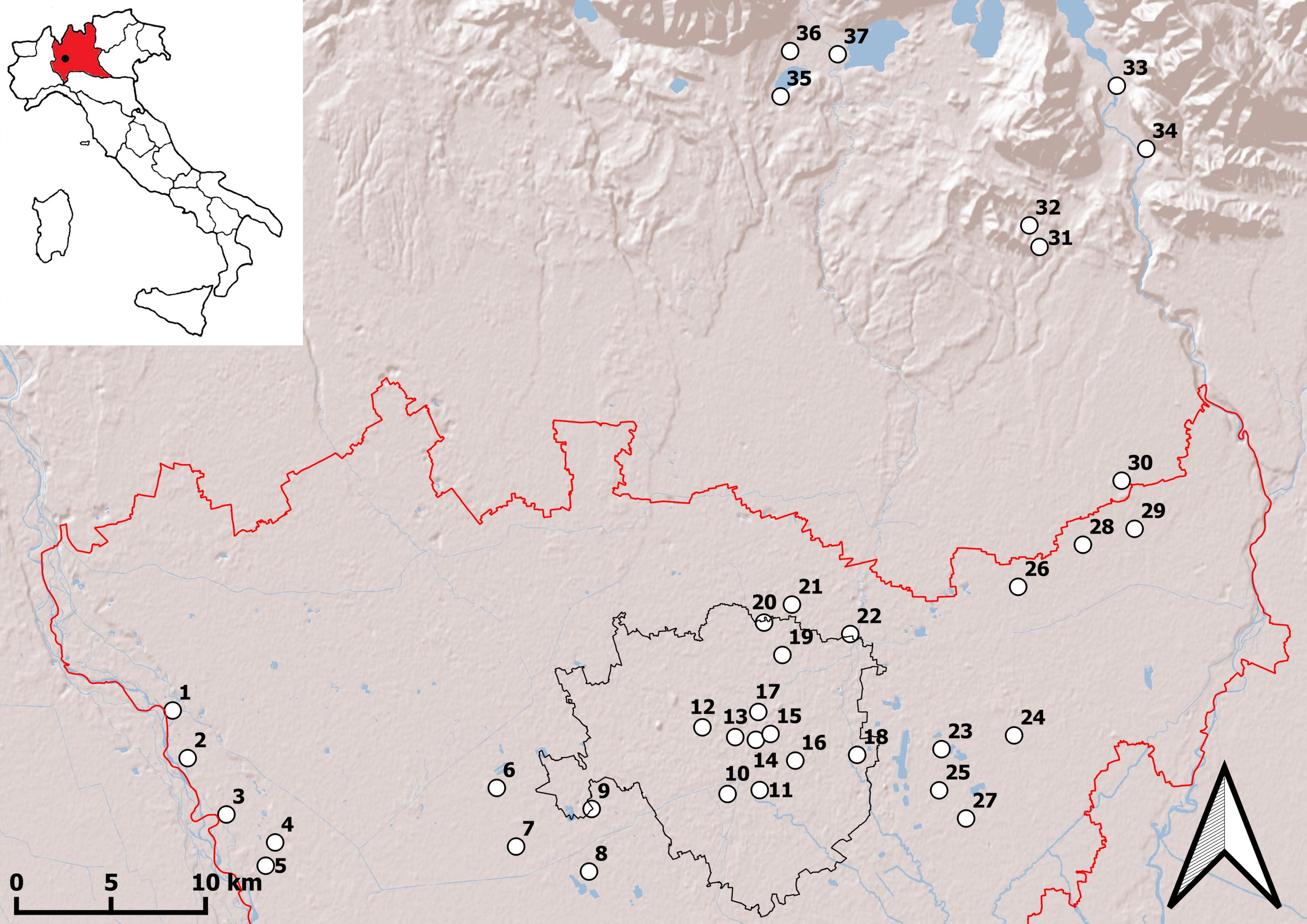
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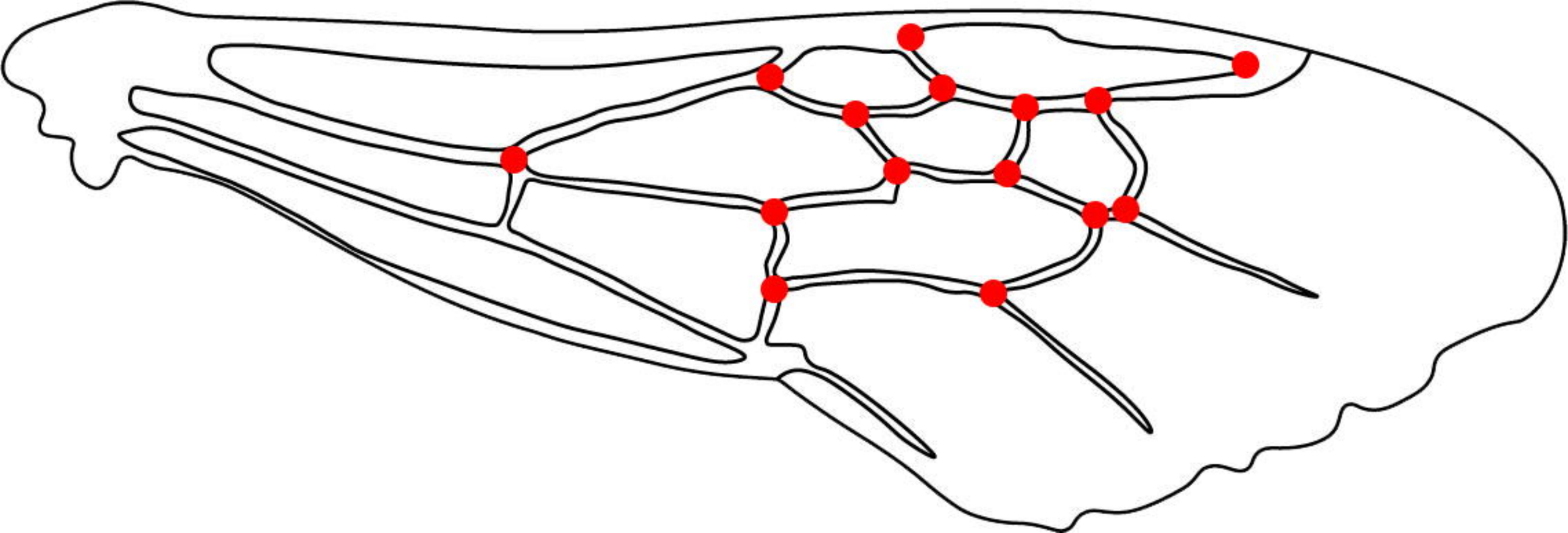

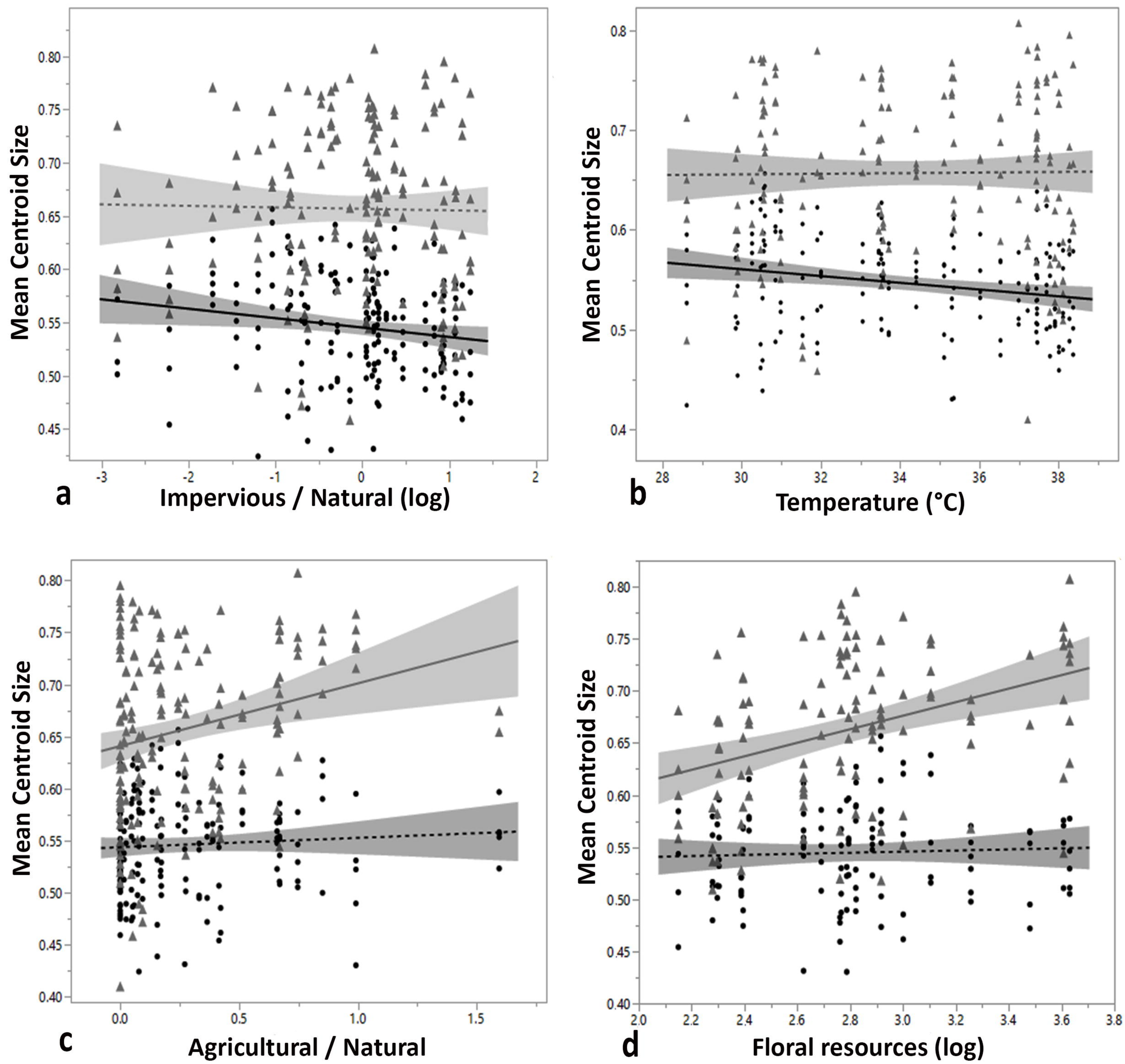
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a
Impervious / Natural (log)
Mean Centroid Size
0.8
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b
Temperature (°C)
Mean Centroid Size
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Agricultural / Natural
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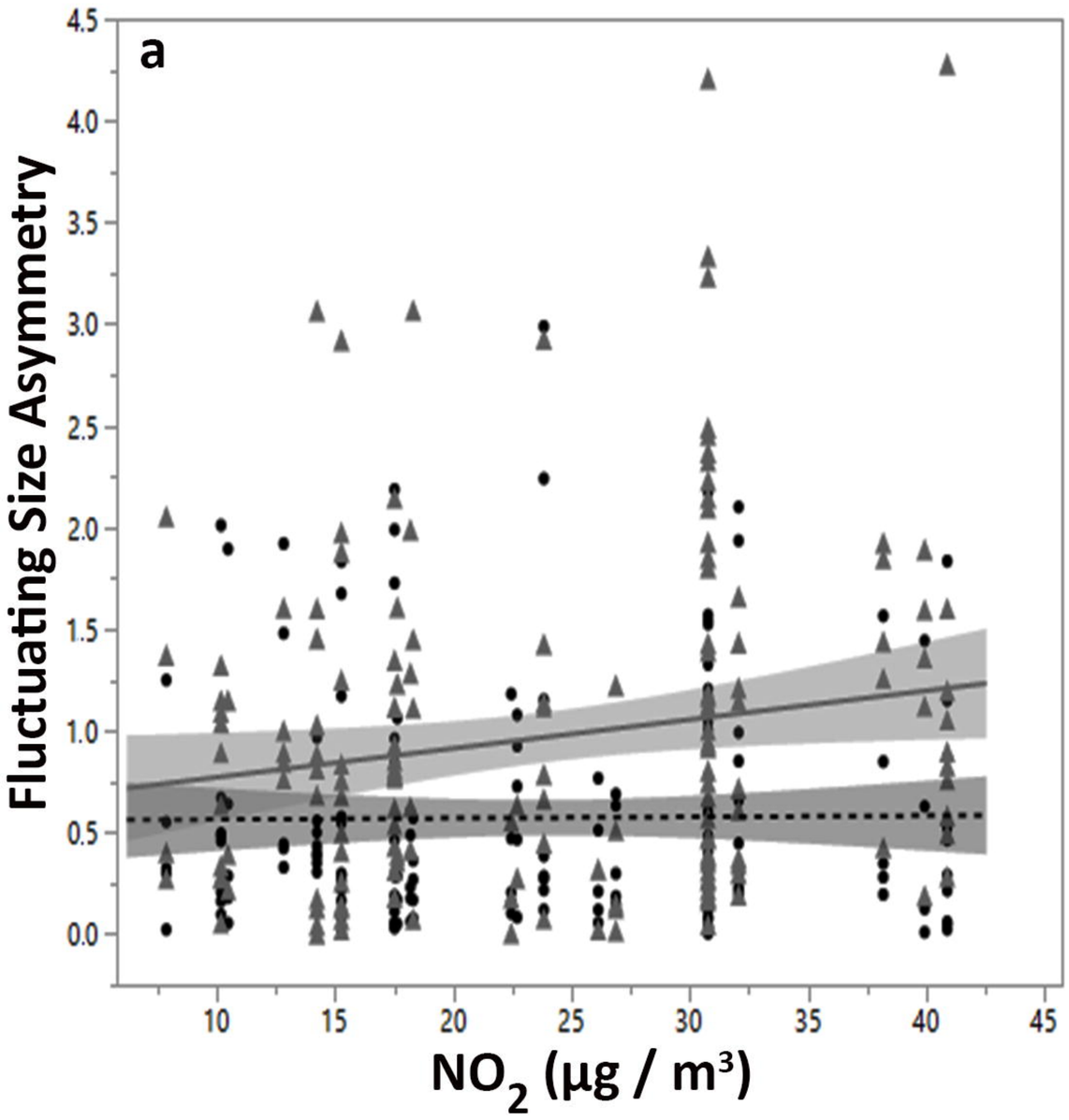
a
Fluctuating Size Asymmetry
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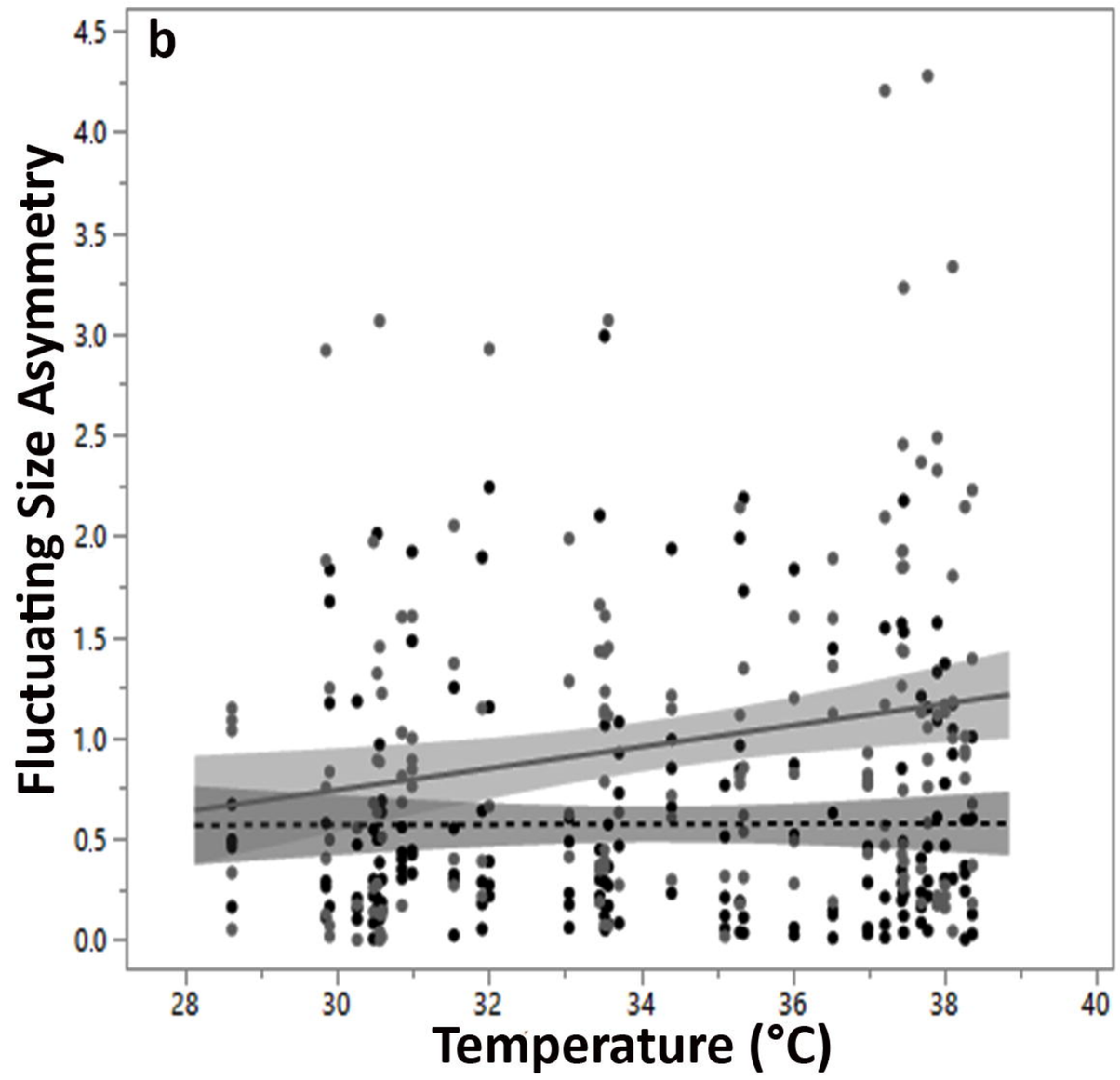
NO2 (µg / m³)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Fluctuating Size Asymmetry
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Temperature (°C)