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Wing shape patterns among urban, suburban, and rural populations of *Ischnura elegans*
(Odonata: Coenagrionidae)

Giovanna Villalobos-Jiménez* & Christopher Hassall^a

^a*School of Biology, University of Leeds, Woodhouse Lane, Leeds UK, LS2 9JT*

*Corresponding author. E-mail: villalobos.giovanna@gmail.com

Abstract

Dragonflies and damselflies (the Odonata) are among the most efficient flying insects. However, fragmentation of the landscape can increase distance between habitats and affect costs of dispersal, thus shaping phenotypic patterns of flight-related traits, such as wing shape, wing loading and wing size. Urban landscapes are highly fragmented, which limits dispersal among aquatic habitats. Hence, strong selective pressures can act upon urban populations in favour of individuals with increased flight performance or may lead to the reduction in dispersal traits. Here, we explore differentiation in morphological flight-related traits among urban, suburban, and rural populations of the damselfly *Ischnura elegans*, which is one of the most abundant species in both urban and rural ponds in Europe. We sampled 20 sites across Leeds and Bradford, UK, in an urban-to-rural gradient from June to August 2014 and 2015 ($N_{\text{males}}=201$, $N_{\text{females}}=119$). We compared wing shape among different land use types using geometric morphometrics. Other wing properties analysed were wing aspect ratio, wing loading, and wing centroid size. Unexpectedly, no significant effect of urban land use was found on wing shape. However, wing shape differed significantly between males and females. Additionally, females showed significantly larger wing centroid sizes ($P < 0.001$), increased

wing loading (fore wings: $P = 0.007$; hind wings: $P = 0.002$) and aspect ratio ($P < 0.001$) compared to males across all land use types. Possible mechanisms driving these results are further discussed.

Keywords: Odonata, dragonfly, *Ischnura elegans*, flight performance, geomorphometrics, urban, city, aspect ratio, wing loading, wing size

Introduction

As aerial predators, dragonflies and damselflies (the Odonata) are adapted to fly efficiently in order to catch prey in the air, and also use their flight ability to disperse, migrate, mate, defend territories, and flee from predators (Marden, 2008). Many morphological traits dictate the flight performance in odonates and other insects, such as wing shape and size, body size, wing loading (i.e. the ratio of body mass to wing area), and thoracic muscle mass (Dudley, 2000; Marden, 2008). Body size and muscle mass influence flight performance strongly, since smaller individuals may need less energy to move and show increased agility (Serrano-Meneses, Córdoba-Aguilar, Méndez, Layen, & Székely, 2007), although their muscle mass and fat reserves are more limited (Marden, 1989; Samejima & Tsubaki, 2010; Serrano-Meneses et al., 2007), therefore representing a trade-off between flight performance and energetic costs (Serrano-Meneses et al., 2007). However, for this study, we will focus on the main functional organs for flight: the wings.

Insect wings operate in a different way to that of vertebrates mainly due to their small size. The Reynolds number (a measure used to predict the flow patterns of fluids) of an insect

wing is relatively low, ranging from 10 to 10^4 (Wang, 2005). As the Reynolds number decreases in a wing, its ability to produce lift also decreases (Cheng & Sun, 2016). Hence, larger wings produce more lift and carry heavier loads (Marden, 1987; Wootton, 1992). Wing size also determines to a great extent the wing shape in odonates, with larger odonates having a larger area concentrated at the wing base, compared to smaller odonates with larger concentration of area in the wing apex (David Outomuro, Adams, & Johansson, 2013). However, size and shape can function as independent components of wing morphology (Debat, Bégin, Legout, & David, 2003). The ratio of wing length to width, known as aspect ratio, has also a strong influence on flight performance, although the resulting effect in insects can be quite different to that in vertebrates. In vertebrates, higher aspect ratio (i.e. longer, narrower wings) decreases the lift-to-drag ratio, which in turn decreases energetic costs and facilitates gliding and long-distance dispersal (Norberg, 1989). High aspect ratios have been found in a wide range of migratory birds (Mönkkönen, 1995). On the other hand, lower aspect ratios (shorter, wider wings) improve manoeuvrability (Norberg, 1989), a trait that is particularly important when escaping predators, catching prey, or fighting against a rival male. However, studies regarding the role of aspect ratio in insects have found rather contrasting results. Firstly, given that insects operate at very low Reynolds numbers, it has been proposed that the benefits of higher aspect ratios are in fact reversed in such cases (Wootton, 1992) so that long, narrow wings increase manoeuvrability, whereas short, wide wings allow long-distance movements with low energetic costs. However, marginal populations of *Calopteryx splendens* and *Coenagrion puella* – both damselflies with expanding range margins – have shown longer, narrower wings, which may suggest that higher aspect ratios reduce energetic costs in a similar way to vertebrates, although the costs and benefits have not been demonstrated (Hassall, Thompson, & Harvey, 2008, 2009).

Importantly, aspect ratio is not the only element of wing shape driving flight efficiency. For instance, petiolate or narrow-based wings – such as the wings of many zygopterans – are associated with slow flight and hovering because the base shows low angular velocity and contributes little force, whereas broad-based wings allow a wider range of speeds, from gliding to high-speed flight (Wootton & Newman, 2008). This is particularly true in anisopterans, which have wide-based hind wings that permit efficient gliding and fast flight (Bomphrey, Nakata, Henningsson, & Lin, 2016; Wootton & Newman, 2008). Wing loading is another important factor driving flight performance, it is defined as the ratio of body weight to the total wing area (Dudley, 2000). Wing loading influences the amount of thrust generated per wing beat (Dudley, 2000) and increases flight speed and lift (Alerstam, Rosén, Bäckman, Ericson, & Hellgren, 2007).

Certain species have shown adaptations in these morphological traits to improve flight performance in order to disperse to environments of limited access, e.g. Taylor and Merriam (1995) show that wing shape of *Calopteryx maculata* is affected by habitat fragmentation. More recently, Outomuro, Dijkstra, & Johansson (2013) show that habitat type influences wing shape of *Trithemis* spp., with long, narrow wings being more abundant in forested areas.

Urban environments represent heavily fragmented habitats, with low vegetation cover and frequent barriers such as roads and buildings (Forman, 2014; Grimm et al., 2008), all of which may impede dispersal to other aquatic habitats. Population genetic studies have shown that urban odonate populations are genetically isolated from rural populations (Sato, Kohmatsu, Yuma, & Tsubaki, 2008; Watts, Rouquette, Saccheri, Kemp, & Thompson, 2004).

Hence, the strong selective pressures acting upon urban odonate populations may drive wing patterns in favour of individuals with increased flight performance. Such a result has been found in the damselfly *Coenagrion puella* (Tüzün, Op de Beeck, & Stoks, 2017), although this study only analysed phenotypic patterns among urban and rural males.

Here, we study the intraspecific variation in wing size, shape and wing loading of the damselfly *Ischnura elegans* in an urban-to-rural gradient to investigate which wing pattern is more successful in urban areas where dispersal may be limited. This species shows slow-speed flight (Bomphrey et al., 2016) and the mean distance of dispersal is less than 200 m, but the females are more likely to disperse and, therefore, more likely to move from their breeding site (Conrad et al., 2002). However, it is widely distributed in Europe and is abundant in urban and rural wetlands (Goertzen & Suhling, 2013), presumably as a result of being able to colonise new habitats and withstand hostile conditions associated with urban environments (Villalobos-Jiménez, Dunn, & Hassall, 2016). Considering this species presents heavily petiolated wings, which generate considerable lift distally, this species may benefit from having longer wings by increasing flight efficiency and reducing energetic costs (Bomphrey et al., 2016; Dudley, 2000; Wootton & Newman, 2008). Hence, we hypothesize that large wings with high aspect ratios will be more abundant in urban areas to decrease the energetic costs of long-distance dispersal, whereas in rural areas, the smaller wings with low aspect ratios will be predominant due to the facilitation of movement in the highly vegetated areas. Suburban populations will show an intermediate form of wing shape between urban and rural populations.

Materials and methods

Field survey

We surveyed 20 ponds across Leeds and Bradford (Table 1; Figure 1) in an urban to rural gradient from June to August 2014 and 2015. Classification of sampling sites was according to the proportion of urbanisation within 1 Km around each pond, which was calculated using the 25m Land Cover Map 2007 (Centre for Ecology & Hydrology, www.ceh.ac.uk/services/land-cover-map-2007) in ArcGIS 10.1 (ESRI, 2011). The LCM2007 data categorises land use into 23 classes, including two different classes for urban and suburban land types. Because morphometric traits have shown small-scale spatial variation (D. Outomuro et al., 2013; Taylor & Merriam, 1995), sites were classified in three categories: urban, suburban, and rural. Sites with an urban cover of >45% in a 1 Km buffer were classified as urban; sites with a suburban cover of >45% in a 1 Km buffer were classified as suburban, and sites with an urban and suburban cover of <45% in a 1 Km buffer were classified as rural. Urban areas are typically heavily fragmented (Grimm et al., 2008), therefore sites classified as “urban” in this study are expected to be strongly fragmented compared to the “suburban” areas (moderately fragmented) and the “rural” areas (low fragmentation). However, the configuration of the landscape (i.e. fragmented vs continuous) was not directly assessed in this study. A total of 320 specimens of *Ischnura elegans* ($N_{\text{males}}=201$, $N_{\text{females}}=119$) were collected with a net, which were then fixed with 70% ethanol.

Geomorphometric analysis

Wings were dissected from the body as close to the thorax as possible and mounted on matte adhesive tape. Wings were scanned using the slide scanner on a Canon CanoScan LiDE 25 flatbed scanner with fixed exposure at 1200dpi. Left wings were reflected so that both left and right wings had the same orientation. After dissecting the wings, the specimens were weighted to the nearest 0.1 mg to obtain body mass.

In order to analyse wing shape, we used geometric morphometrics, which uses a multivariate approach to estimate the qualitative properties of wing shape and transform them to a quantitative measure (Bookstein, 1991). We digitised nine type 1 landmarks (Bookstein, 1991) defined as the intersection between the main veins (Figure 2) according to Arambourou, Sanmartín-Villar, & Stoks (2017) using the geomorph package (Adams & Otárola-Castillo, 2013) in R 3.4.0 (R Core Team, 2013). As a proxy of wing size, the wing centroid sizes were calculated as the square root of the summed squared distances from each landmark to the geometric centre of each wing (Bookstein, 1991). We also quantified (1) wing area as the area of the polygon defined by the landmarks 1–8, and (2) wing length as the distance between landmarks 1 and 4 (Arambourou et al., 2017). Wing loading was calculated as body mass/wing area and wing aspect ratio was calculated as wing length²/wing area (Berwaerts, Van Dyck, & Aerts, 2002; Gilchrist, Azevedo, Partridge, & O'Higgins, 2000). Body mass was calculated after removing the wings, therefore wing loadings here did not include wing mass. Both wing length ($r = 0.99$, $P < 0.001$) and area ($r = 0.96$, $P < 0.001$) were highly correlated with wing centroid size.

Wing shape was estimated using the landmarks previously digitised, with any differences among wing configurations other than shape differences (i.e., position, orientation and size) removed using the generalized Procrustes superimposition method (Rohlf & Slice, 1990). To confirm that wing shape is independent of wing size, or in other words, that there were no allometric effects, we calculated a linear regression with the Procrustes coordinates as the response variables and wing centroids as the explanatory variable. To assess the significance of this relationship, 100 permutations were conducted by randomly reassigning observations for Procrustes coordinates to observations for centroid sizes. We found a significant allometric effect ($F_{1, 1224} = 141.7, P = 0.009$), as has been observed previously in insect wings (Debat et al., 2003; Gilchrist et al., 2000). Because the shape variables were strongly dependent on wing centroids, the residuals of this regression were used as the non-allometric shape component. Principal components analysis (PCA) was then carried out on the non-allometric landmarks to obtain the shape variables as principal components scores. Considering the first three PC scores accounted for 83.51% of the overall variation (PC1: 60.58%, PC2: 12.11%, PC3: 10.82%), we used only these as a measure of wing shape.

Statistical analysis

To test the effect of land use on flight related traits, we used a linear mixed model using the lme4 package (Bates, Maechler, Bolker, & Walker, 2014) with each of the flight-related traits as dependent variables (PC1, PC2, PC3, aspect ratio, wing loading, and wing centroids); sex and types of land use (urban, suburban, rural) were the grouping explanatory variables, and the location was added as a random effect to account for spatial variation. Even though flight-related traits have been shown to vary with time (Gyulavári et al., 2017; Stewart & Vodopich, 2018), we could not analyse temporal differences due to low sample sizes (see

Table 1), all data from 2014 and 2015 was pooled for this study. Before running the analyses, we compared the flight-related traits (PC1, PC2, PC3, aspect ratio, wing loading, and wing centroids) among types of wing (left and right, fore and hind wings) using a MANOVA to test whether all wings could be pooled together for the analysis. The results suggested fore and hind wings had significantly different flight-related traits (Wilk's $\lambda = 0.161$, $df_N = 6$, $df_D = 1160$, approximate $F = 1005.8$, $P < 0.001$), but no significant differences were found among left and right wings (left and right fore wings: Wilk's $\lambda = 0.99$, $df_N = 6$, $df_D = 574$, approximate $F = 0.814$, $P = 0.56$; left and right hind wings: Wilk's $\lambda = 0.99$, $df_N = 6$, $df_D = 579$, approximate $F = 0.966$, $P = 0.45$). Therefore, only the left wings were used for the analyses, separated by fore wings and hind wings. The P -values were obtained using the Satterthwaite approximation to calculate denominator degrees of freedom. Variation Inflation Factors (VIF) were calculated to account for multicollinearity (see Table S1 in Supplementary Information), and the resulting P -values from the linear mixed models were transformed using the False Discovery Rate (FDR) to account for multiple testing.

Results

Aspect ratio was significantly higher in females than males for both fore and hind wings (Table 2; Figure 3), but no significant differences were observed among land use types (Table 2; Figure 3). Wing loading was also significantly larger in fore and hind wings of females compared to wings of males (Table 2; Figure 3), although wing loadings were not significantly different across land use types (Table 2; Figure 3). Wing centroid size was also significantly larger in fore and hind wings of females than males (Table 2; Figure 3).

Regarding the wing shape variables, all PC scores represented subtle changes in the wing planform (see Figure 4), with very little shape variation ($\sigma^2 = 0.001$). All PC scores were significantly correlated with aspect ratio, although the strength of the correlation was weak except for PC3 (PC1, fore wings: $r = -0.242$, $P < 0.001$; PC1, hind wings: $r = -0.293$, $P < 0.001$; PC2, fore wing: $r = -0.292$, $P < 0.001$; PC2, hind wing: $r = -0.296$, $P < 0.001$; PC3, fore wing: $r = -0.654$, $P < 0.001$; PC3, hind wing: $r = -0.625$, $P < 0.001$). PC1 showed significant differences between sexes (Table 2), with females showing larger PC1 values in both fore (Figure 4; Figure 5) and hind wings (Figure 4(e)). Females also exhibited significantly larger PC2 values than males, but only in hind wings (Table 2; Figure 4; Figure 5). PC3 was not significantly different among sexes (Table 2). No significant effect of land use was found in any of the shape variables (Table 2).

Discussion

In this study, we found no evidence of differentiation of flight-related morphological traits in *I. elegans* due to urbanisation. Instead, there were subtle, but significant differences between males and females (see Figure 4 and Figure 5), particularly females having larger wing centroid sizes in fore and hind wings, higher aspect ratios in fore and hind wings, increased wing loadings in fore and hind wings, higher PC1 values in fore and hind wings, which is associated with an increased distance between the pterostigma and the nodus (see Figure 4), and higher PC2 values in hind wings, which represents a shorter distance between

the nodus and the radial section (Figure 4). In the following text, we will suggest plausible mechanisms driving these responses.

Urban areas can be heavily fragmented (Grimm et al., 2008) and fragmented habitats can affect morphological flight-related traits in odonates (D. Outomuro et al., 2013; Taylor & Merriam, 1995). However, in this study there were no significant differences found in morphological traits associated with flight performance and dispersal among urban, suburban, and rural populations. This result is unexpected since it contrasts with other studies, particularly with Tüzün et al (2017) which found significant differences in flight-related traits in odonate populations due to urbanisation. However, this study only found such differences in flight endurance and in one shape variable (relative warp 3) which only contributed to ca. 9% of variation, the rest of the shape variables did not show significant differences among urban and rural populations. Tüzün et al (2017) reported higher flight endurance in urban males of *Coenagrion puella*, though females were not assessed. However, *C. puella* is more likely to disperse to other ponds compared to *I. elegans* (Conrad et al., 2002), which may explain why the former species showed differences in flight-related traits between urban and rural populations, and the latter did not. Moreover, in this study, we only focused on the morphological differences in flight-related traits, we did not measure flight endurance, flight muscle mass, fat content, metabolic rate, or account for genetic variation, all of which influence flight performance and dispersal (Marden, 2008). Petiolated wings, as mentioned, do not allow gliding, therefore large energy storage and higher flight endurance could be advantageous for long-distance dispersal. Despite the fact that no differentiation was found in the phenotypic traits of *I. elegans* associated to flight, strong genetic differentiation was found among urban and rural populations of other *Ischnura* species, namely *I. senegalensis* and *I.*

asiatica, which suggests dispersal is indeed limited in the urban landscape, thus isolating urban populations (Sato et al., 2008). Even though the genetic differentiation of urban and rural populations of *I. elegans* has not been investigated, urban populations are expected to be heavily isolated due to increased habitat fragmentation in cities and the low mobility of this species. Further studies are encouraged to explore the intraspecific variation of flight performance, dispersal, and genetic diversity within an urban landscape in *I. elegans* and other species, particularly linking flight morphology and function.

Another plausible reason why we did not find any significant differences in flight-related traits could be due to the increased temperature found in urban areas, known as the urban heat island (UHI) effect (Grimm et al., 2008). Increased temperatures can facilitate dispersal (Boyle, Schwanz, Hone, & Georges, 2016; Chen, Hill, Ohlemuller, Roy, & Thomas, 2011; Lett, Ayata, Huret, & Irisson, 2010; Travis et al., 2013), particularly in odonates given their tropical evolutionary origin (Hassall & Thompson, 2008; Pritchard & Leggott, 1987). As temperature increases, so does the lift force (Samejima & Tsubaki, 2010), wingstroke frequency (May, 1981) and power output of insect flight muscles (Stevenson & Josephson, 1990). This may counteract the negative impacts of fragmentation in urban areas and, therefore, urbanisation overall may not have any considerable impacts on flight performance and dispersal. However, the effects of temperature on dispersal are far more complex. Firstly, not all species benefit from increased temperature since many species have adapted to more temperate conditions (Jaeschke, Bittner, Reineking, & Beierkuhnlein, 2013; Travis et al., 2013). Moreover, temperature interferes with many other factors such as body size (McCauley, Hammond, Frances, & Mabry, 2015) and wing shape, even in the same study species, *I. elegans* (Arambourou et al., 2017), which can affect flight performance. Therefore,

increased temperature does not always have a positive impact. Further research is needed to analyse the effect of dispersal on odonates linking fragmentation and increased temperatures in urban areas.

One of the main findings in this study is the conspicuous distinction of flight-related traits among males and females of *I. elegans* (Figure 5). Sexual wing dimorphism has been reported previously in *I. elegans* (Arambourou et al., 2017), and can be attributed to the difference in selection pressures to which males and females are subject to. Both males and females disperse away from their native water body after emerging to feed until they reach sexual maturity (Corbet, 1999). However, after becoming sexually mature, females only return to breeding sites when they are receptive (Corbet, 1999) and undergo stronger selective pressure to forage (Anholt, 1992), whereas males tend to stay close to breeding sites in order to find a mate (Beirinckx, Van Gossum, Lajeunesse, & Forbes, 2006; Corbet, 1999), therefore females may need to be more dispersive than males (Beirinckx et al., 2006). Moreover, females can also show impaired locomotor performance once they become gravid (Carlson, McGinley, & Rowe, 2014; Olsson, Shine, & Bak-Olsson, 2000; Samietz & Köhler, 2012; Shine, 2003) and in flying organisms, pregnancy has shown to affect take-off negatively (Almbro & Kullberg, 2012; Lee, Witter, Cuthill, & Goldsmith, 1996; Veasey, Houston, & Metcalfe, 2001). In such case, the larger, longer, and narrower wing pattern found in females could be of aid for long-distance dispersal, since the angular velocity increases towards the wing tip in flapping wings, notably in petiolated wings where the velocity gradient from base to tip is more pronounced (Bomphrey et al., 2016; Dudley, 2000), thus providing more lift per wingbeat (Bomphrey et al., 2016). The increased wing loadings found in females also promote dispersal by increasing the thrust generated per wingbeat (Dudley, 2000).

Interestingly, PC3 showed no statistically significant differences among males and females, even though in Figure 4 there is a consistent sex-dependent pattern. Additionally, PC3 and aspect ratio were strongly correlated, so significant differences would be expected. Perhaps this is due to the fact that overall wing shape showed very little variation ($\sigma^2 = 0.001$), and PC3 represents only little over 10% of this variation, therefore the changes represented by PC3 may be too subtle to affect dispersal. However, more studies are needed to confirm the effect of increased aspect ratio in the flight performance of *I. elegans*.

Urbanisation is one of the main drivers of ecosystem change (Faeth, Bang, & Saari, 2011; Grimm et al., 2008; McKinney, 2008), and the resulting fragmentation of the landscape can shape phenotypic patterns to facilitate dispersal (e.g. Tüzün et al., 2017). However, *I. elegans* is a widespread species and one of the most abundant damselflies in Europe (Dijkstra & Lewington, 2006; Dow, 2010), which can tolerate a wide range of stressors (Dow, 2010). The results of this study suggest that this species' wing morphology is not affected by increased fragmentation in the urban landscape at the scale studied and may also explain why this species thrives in urban ponds (Goertzen & Suhling, 2013). However, more studies are needed to analyse intraspecific and interspecific variation of other flight-related traits, such as flight endurance, flight performance, fat content and, ultimately, dispersal success in order to disentangle the effects of urbanisation at a population and community level. This, in turn, will allow a better understanding of which species are more susceptible to land use change and how organisms and communities respond to novel environments.

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Tables

Table 1. Sampling site locations, land use classification of each site, percentage of urban (URB), suburban (SUBURB), woodland (WOOD), and grassland (GRASS) cover in a 1 Km buffer around the sampling sites, and sample sizes of fore wings (N_{FW}) and hind wings (N_{HW}) of males and females of *Ischnura elegans* per site and per year.

Location	Latitude	Longitude	Land use	URB	SUBURB	WOOD	GRASS	2014				2015			
								Females		Males		Females		Males	
								N_{FW}	N_{HW}	N_{FW}	N_{HW}	N_{FW}	N_{HW}	N_{FW}	N_{HW}
ASD metal services FGH Ltd Listerhills Warehouse	53.767975° N	1.512972° W	urban	45.80	23.55	4.25	10.47	0	0	0	0	10	9	17	17
Kemira Chemicals UK Skelton Grange Environment Centre	53.797836° N	1.772692° W	urban	62.79	35.09	0.00	2.12	0	0	0	0	9	9	13	11
University of Bradford Main Campus	53.778672° N	1.746394° W	urban	45.35	43.09	3.39	8.17	0	0	0	0	26	24	23	23
Harold Park	53.776439° N	1.499625° W	urban	49.02	14.06	4.47	17.31	0	0	0	0	6	8	4	3
Hollin Lane	53.793328° N	1.764911° W	urban	64.78	33.05	0.00	2.16	0	0	0	0	4	4	2	3
Horton Bank Country Park	53.7581° N	1.773817° W	suburban	11.47	67.87	0.15	18.72	6	5	11	12	4	4	4	4
Primrose Valley Park	53.828492° N	1.571503° W	suburban	7.60	64.08	10.38	13.85	0	0	8	8	2	2	17	18
Reevy Mill Dam	53.774464° N	1.810864° W	suburban	7.62	66.07	2.95	22.22	0	0	0	0	21	20	23	23
Bierley Hall Wood Farnley Hall Fish Local Nature Reserve	53.802242° N	1.46535° W	suburban	12.34	62.43	2.54	22.32	15	14	18	17	2	1	4	4
Letchmire Pastures Nature Reserve	53.764944° N	1.790764° W	suburban	10.95	80.29	0.00	8.76	0	0	0	0	2	2	6	6
Middleton Park	53.759392° N	1.737078° W	rural	13.43	25.12	5.17	52.48	7	8	16	16	3	3	2	1
None Go Bye Farm	53.788094° N	1.622917° W	rural	8.16	28.72	11.66	33.08	6	6	6	6	0	0	1	2
Paul's pond	53.774514° N	1.348325° W	rural	1.04	3.83	3.94	15.71	8	10	8	8	0	0	0	0
Roundhay Park	53.740858° N	1.357344° W	rural	5.33	5.93	4.97	26.32	6	6	13	12	0	0	0	0
Swillington Organic Farm	53.7543° N	1.546175° W	rural	2.18	31.46	28.36	31.66	6	6	28	34	31	28	41	38
Woodhall Lake	53.871872° N	1.64115° W	rural	2.10	6.97	5.29	75.06	6	6	12	12	0	0	4	3
	53.866856° N	1.603722° W	rural	0.28	12.80	9.36	71.97	6	6	23	23	3	3	14	17
	53.840606° N	1.497186° W	rural	4.20	21.54	17.23	39.46	2	2	4	8	11	12	23	25
	53.757781° N	1.4336° W	rural	2.64	10.29	16.55	33.79	12	12	13	14	0	0	0	0
	53.807097° N	1.695217° W	rural	8.43	37.10	5.27	43.36	2	1	7	7	0	0	0	0

Table 1. Results of the linear mixed-effects models testing the effect of land use and sex on the PC scores, wing aspect ratio, wing loading, and wing size of fore and hind wings. P-values were obtained using the Satterthwaite approximation to calculate denominator degrees of freedom. P-values presented here have been adjusted for multiple testing using the FDR correction. Significant differences are in bold.

	NumDF	Fore wings			Hind wings		
		DenDF	<i>F</i>	<i>P</i>	DenDF	<i>F</i>	<i>P</i>
<i>PC1</i>							
Sex	1	275.82	90.083	<0.001	286.38	103.179	<0.001
Land use	2	11.41	1.562	0.335	12.04	1.635	0.332
<i>PC2</i>							
Sex	1	277.83	3.149	0.154	285.15	17.613	<0.001
Land use	2	15.85	4.498	0.068	12.74	1.935	0.277
<i>PC3</i>							
Sex	1	277.02	2.061	0.277	285.06	1.801	0.277
Land use	2	15.66	1.120	0.407	17.98	0.028	0.972
<i>Wing aspect ratio</i>							
Sex	1	277.77	82.614	<0.001	286.31	65.776	<0.001
Land use	2	15.07	3.119	0.154	11.06	1.406	0.361
<i>Wing loading</i>							
Sex	1	266.35	9.202	0.007	275.40	11.439	0.002
Land use	2	15.23	1.106	0.407	15.18	1.021	0.419
<i>Wing size</i>							
Sex	1	268.23	394.155	<0.001	278.27	316.301	<0.001
Land use	2	14.90	2.025	0.277	15.26	0.893	0.448

Figures

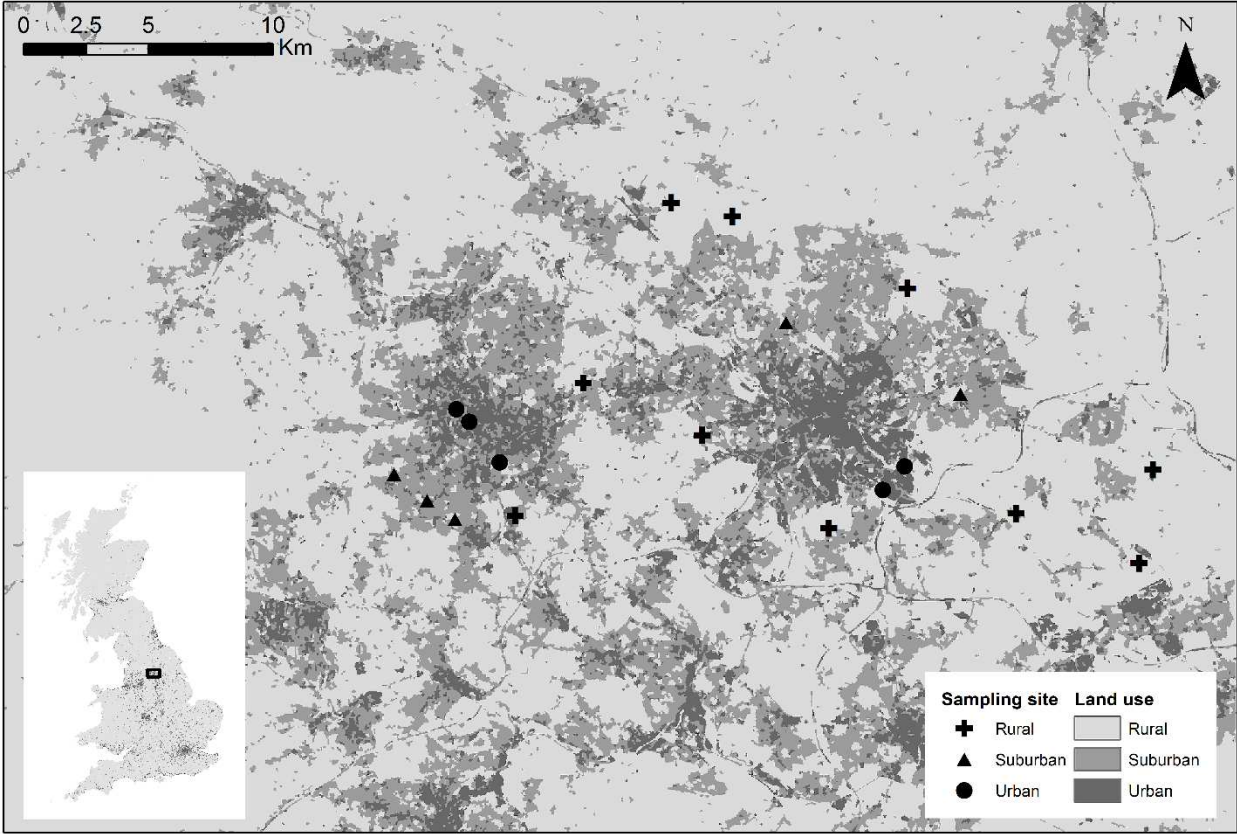


Figure 1. Map of the sampling sites for the morphometric analysis in Leeds (right) and Bradford (left), indicating the degree of urbanisation and classification of the sampling sites.

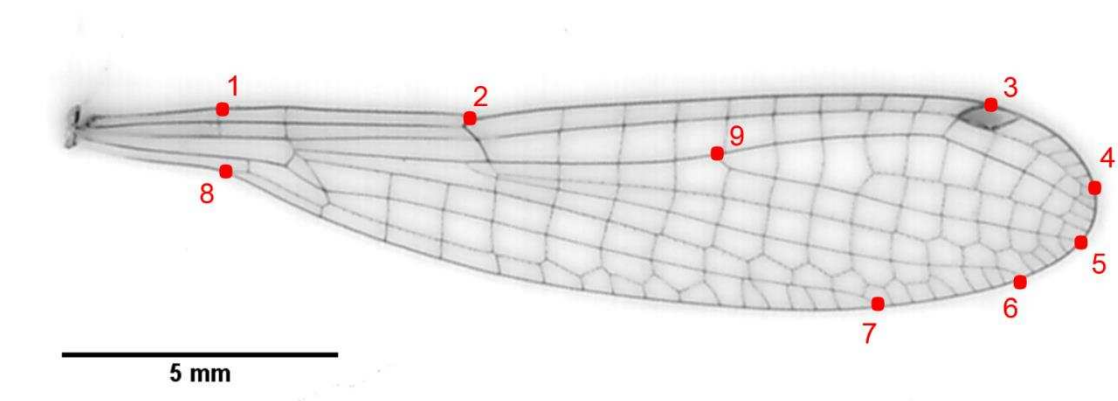


Figure 2. Localization of the nine landmarks digitised on the wings of *Ischnura elegans* according to Arambourou et al. (2017).

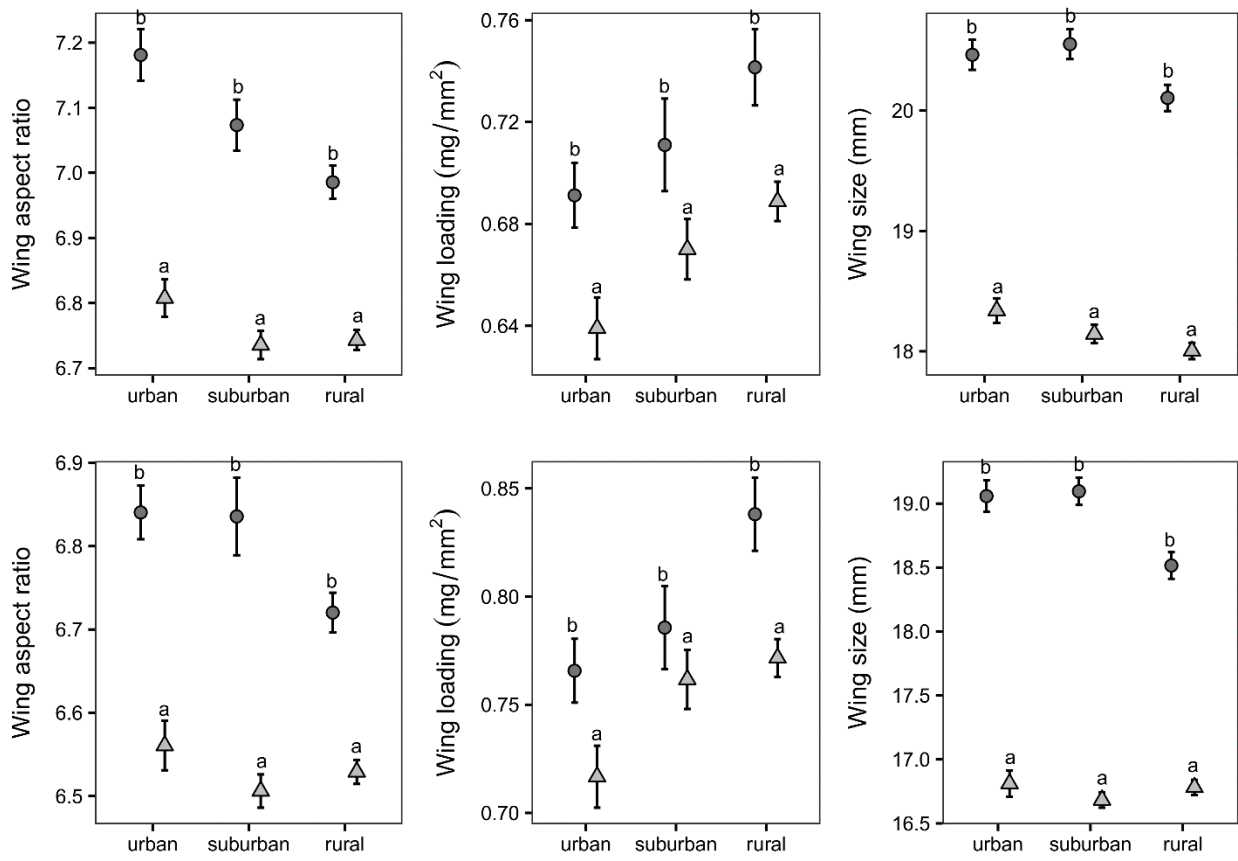


Figure 3. Mean wing aspect ratio (*left panel*), wing loading (*middle panel*) and wing centroid size (*right panel*) of males (*triangle*) and females (*circle*) from the urban, suburban, and rural populations sampled. Fore wings are represented on the top panel, hind wings on the bottom panel. Annotations represent significant differences. Error bars represent ± 1 SE.

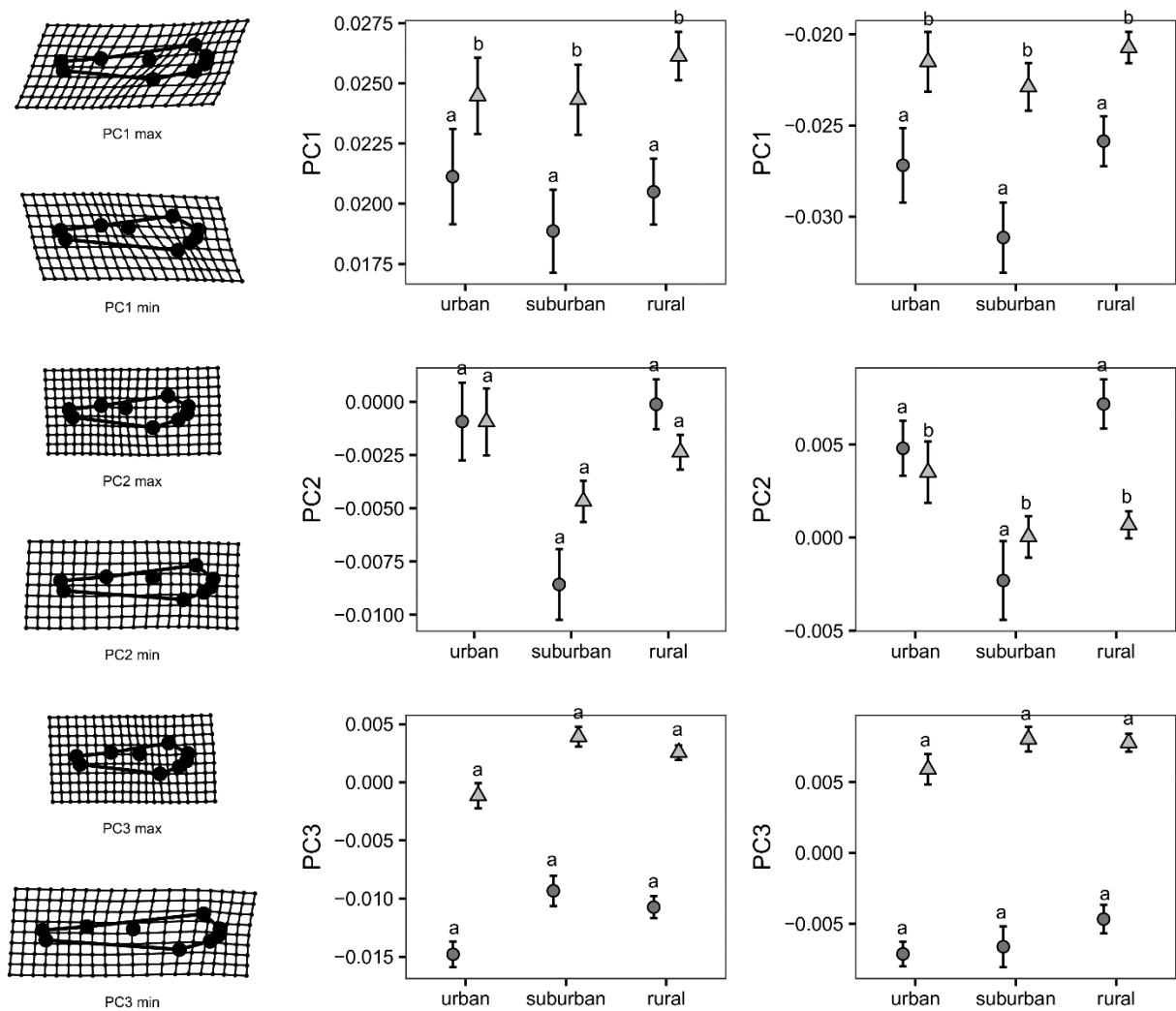


Figure 4. Results of the geometric morphometric analysis showing mean scores of PC1 (*top panel*), PC2 (*horizontal middle panel*) and PC3 (*bottom panel*) by males (*triangle*) and females (*circle*) from the urban, suburban, and rural populations sampled. On the left panel, the deformation grids showing the changes in mean shape represented by the maximum and minimum values of the PC scores. The PC scores in fore wings are depicted on the vertical middle panel, and on the right panel, the PC scores in hind wings. Annotations represent significant differences. Error bars represent ± 1 SE. To better visualize shape variations on the deformation grids, an amplification factor of 2 was applied.

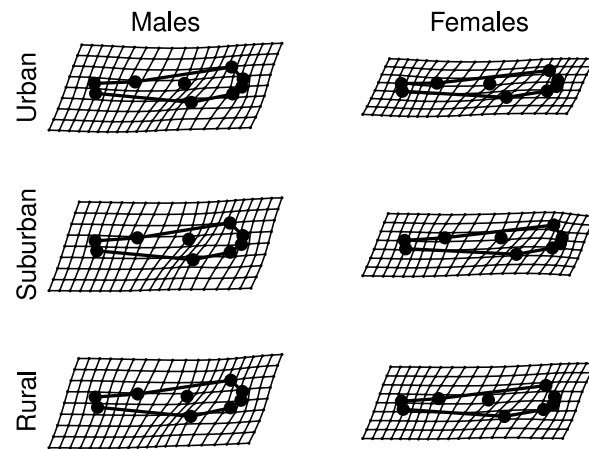


Figure 5. Deformation grids showing the mean wing shape of males and females in urban, suburban, and rural populations. To better visualize shape variations, an amplification factor of 5 was applied.