Eco-evolutionary dynamics in urbanized landscapes: Evolution, species sorting and the change in zooplankton body size along urbanization gradients — Brans et al., 2016 — doi: 10.1098/rstb.2016.0030

SUPPLEMENTARY INFORMATION A - Material and Methods

Data acquisition

Habitat selection

We sampled 84 permanent shallow ponds along urbanization gradients in Flanders (Belgium; latitude 51° 00' 00" N, longitude 4° 30' 00" E) in the period May - July 2013 (Fig. SA1). Before sampling, sample sites were selected based on percentage built-up area (%BA, i.e. surface area taken by buildings, houses and industrial infrastructure; derived from GRBgis maps and "Ground Building" values, Flanders Geographical Information Agency) and involved gradients in urbanization along the cities of Antwerpen, Brussel, Gent and Leuven. Built-up area is a very restricted measure of urbanization as it excludes roads, parking lots etc. As a result, even Gbg values as low as 10 % represent already high levels of urbanization. Ponds were selected in squared plots of both 200 × 200 m and 3 × 3 km such that they represented an approximately equal number of ponds with estimated low ("rural"), intermediate and high urbanization levels in both the immediate surroundings of the ponds (200 × 200 m squared plots) and at a larger spatial scale (3 × 3 km squared plots). After sampling, the degree of urbanization was more precisely quantified for each pond at seven separate radii around the pond (50, 100, 200, 400, 800, 1600, and 3200 meter) based on percentage built-up area. Assessing urbanization at different independent spatial scales allows to disentangle possible scale-dependent effects (e.g. a local "park cooling" effect versus the more regional "urban heat island effect"). After sampling, temperature loggers were installed in two urban and two rural ponds, at a depth of 10-20 cm below the water surface, to monitor water temperature on a daily basis (time intervals of 15 minutes) throughout the year (2013-2014).

Zooplankton community composition and intraspecific trait variation for body size

From each pond, we took depth-integrated water samples using a tube sampler in both pelagic and littoral sites. Depending on zooplankton densities, 20 up to 40 L water was filtered over a 64 μm sieve; samples were fixated in 4 % formalin and stored in 60 mL vials until species identification and body size measurements. A minimum of 300 cladoceran zooplankton individuals per sample were identified to species level following Amoros ([1]) and Flössner ([2]). Species densities were expressed as number of individuals per liter. Samples containing less than 300 individuals were fully counted. For each species present in a sample, 15 random individuals were measured to assess the average local species body size. These measurements included juveniles as well as adults. For samples containing *D. magna*, an additional set of 15 adults was measured to also calculate average adult body size of this focal species. A list of species present across all ponds and their mean body size are given in Table SA1; the number of species in each pond is given in Table SA2.

Genetic variation and phenotypic plasticity in body size in Daphnia magna

Twelve *Daphnia magna* populations inhabiting ponds along the urbanization gradient as

characterized by percentage built-up area at a radius of 3200 m were sampled for a common garden experiment. We isolated six random lineages from each population and kept them in the laboratory as clonal lineages. The experimental animals were exposed to two temperatures, 20 °C and 24 °C (with 20 °C reflecting the average July maximum temperature in rural ponds observed between 7/17/14 and 7/31/14; Table SA3, Fig. SA2) to mimic the temperature gradient along the urbanization gradient. With three replicates per lineage × treatment combination, this resulted in 12 populations × 6 lineages × 2 temperatures × 3 replicates = 432 experimental units. To obviate interference from conditions in the source habitat through (grand)maternal effects, we grew all 432 lines for a minimum of two generations individually in 100 mL vials under standardized laboratory conditions (water baths at 20 ± 0.6 °C, 14:10h L:D photoperiod, dechlorinated tap water). Animals were fed daily ad libitum with the green algae *Desmodesmus obliquus* (daily restored to a concentration of 1×10^5 cells mL^{-1}), and 80 % of the medium was refreshed every other day. Neonates <24h old from the second to fourth clutch were used as experimental animals and inoculated in cohorts of 12 individuals in 500 mL jars placed in temperature controlled water baths (20 ± 0.6 °C or 24 ± 0.4 °C, with a 14:10h L:D photoperiod and a feeding and medium refreshment regime as described above. To avoid temperature fluctuations, dechlorinated tap water was placed at the appropriate temperature for at least three hours before its use as medium. Of each cohort, four individuals in their first adult instar were measured to score size at maturity (Olympus SZX12, Olympus optical co., LTD; from top of the eye to the base of the tail spine). To assess whether all lineages were genetically unique, clonal identity was determined by screening variation at 27 microsatellite markers, structured in four multiplexes following Jansen et al. ([3]). Genomic DNA extraction was performed using the Proteinase K digestion method as described in Mergeay et al. ([4]), and the Qiagen Multiplex PCR kit (Qiagen, Netherlands) was used for DNA amplification. Microsatellite alleles were scored on an ABI PRISM 3031 automated sequencer (Applied Biosystems) and analysed with Gene Mapper (Applied Biosystems, Liz500 size standard). Lineages that were identical at all loci were considered to belong to the same clone and their data was pooled, leading to a total of 348 experimental observations. Information on pond location, urbanization level, and the number of clones used in our experiment can be found in Table SA4.

Statistical analysis

Data

Because exploratory analyses indicated that patterns of change in body size in the cladoceran communities along the urbanization gradients differed depending on whether the communities were dominated by small- or large-bodied taxa (see Results in main text), we divided the data into two subsets: the large-species dominated community subset and the small-species dominated community subset. The large-species dominated community subset ($n = 34$) involved all ponds with zooplankton communities having more than 5 % of large species in terms of abundances. The large species (average body size >1 mm, see e.g. [5]) were *Daphnia magna*, *D. obtusa*, *D. pulex* and the chydorid *Eurycercus lamellatus*. A 5% cut-off value is inspired by the fact that their body size is large, so that in terms of biomass their contribution is more substantial. We explored analyses with other cut-off values (10 % and 15 %) and these in essence yield the same results. The small-species dominated community subset involved all other ponds ($n = 50$). All analyses on communities presented further were done on the two subsets separately as well as on the total

dataset. For the analyses that also involved genetic variation in *D. magna*, only the large-species subset was used as community dataset. All data analyses were conducted with the R software version 3.2.3 for Windows ([6]). Outliers and influential data points were detected using the Cook's distance, the outlierTest function ('car' package, [6]), and visual screening by plotting the model residuals versus leverage and plotting the data points. The justification for outlier removal as well as the results of analyses without outlier removal are described in Supplementary Information B.

Quantifying the relative importance of intraspecific trait variation (ITV) and interspecific trait turnover (SPT) on the change in cladoceran community body size
We assessed the local average community body size in two different ways. First, we calculated the local community body size for a community j as the abundance-weighted average of the local body sizes of all species present in the local community (i.e. $\bar{z}_L^j = \sum_i q_{ij} z_{ij}$ with q_{ij} the relative abundance and z_{ij} the local average body size value of species i of community j). Second, we calculated it as the abundance-weighted average using the metacommunity-wide average body size of all species (i.e. the average body size of a species across all communities where it is present; $\bar{z}_{MW}^j = \sum_i q_{ij} \bar{z}_i$ with q_{ij} the relative abundance of a species i of community j and $\bar{z}_i$ the metacommunity-wide average body size value of species i).

We quantified the contribution of variation explained by ITV and SPT to the total trait variation along the urbanization gradients using the variation partitioning method described by Lajoie & Vellend ([7]). We built three regression models. In the SPT+ITV model, the abundance-weighted average community body sizes using the local species body size distributions for each community was regressed against percentage built-up area. This model reflects the effect of both intraspecific trait variation of each species and species turnover along the gradient. In the SPT model, the abundance-weighted average community body sizes using the metacommunity-wide species body sizes were regressed against percentage built-up area. This model only accounts for the changes in the relative abundances of species and species replacements along the gradient. Determining the effect of ITV on community trait turnover along the gradient was then done by subtracting the SPT model from the SPT+ITV model, i.e. $\bar{z}_L^j - \bar{z}_{MW}^j = \sum_i q_{ij}(z_{ij} - \bar{z}_i)$. The three regression models were evaluated at all seven spatial scales and for the three different sets of communities (i.e. full dataset, large-species dominated and small-species dominated subset). Based on the Akaike Information Criterion (AIC) we decided to log-transform percentage built-up area for all models. To better meet the assumption of normality for the regression analyses, the abundance-weighted average community body sizes were also log-transformed. One extreme outlier was detected in the ITV model and removed from the full dataset ($n = 83$; see Fig. SA3a,b). For the subset of communities dominated by large species two outliers were removed ($n = 32$; see Fig. SA3e,f), and for the subset of communities dominated by small species one outlier was removed ($n = 49$; see Fig. SA3c,d). Bootstrap analysis was performed to assess variation in our estimates of the contribution of ITV and SPT (Fig. 2 main text; solid line). The variance in community weighted means of body size attributable to SPT and to ITV was assessed as the ratio of the regression sum of squares of the SPT model ($\mathrm{SSR_{SPT}}$) or the ITV model ($\mathrm{SSR_{ITV}}$) over the total sum of squares of the model including both SPT and ITV ($\mathrm{SST_{SPT+ITV}}$). This quantifies the contribution of SPT and ITV, respectively, to the total explained variance in community average body size along

the urbanization gradient. To determine which component, ITV or SPT, has the largest contribution we used the formula $SSR_{ITV}/(SSR_{ITV}+SSR_{SPT})$ ([7]).

Genetic variation and phenotypic plasticity in body size in Daphnia magna
We tested for the effect of urbanization and temperature exposure on genotypic values (see Box SA1) of *D. magna* size at maturity using a set of linear mixed models in which clone (nested in population) and population were included as random effects. Built-up area was log-transformed. Statistical analyses were conducted using the 'lme4' and 'car' packages ([6]) to construct linear mixed models and compute approximate F-test statistics and p-values for fixed effects ([8]). The model was fitted according to the restricted maximum likelihood estimation method (REML), and degrees of freedom calculations for fixed effects were corrected by the Kenward-Roger approximation. Both normality of model residuals and homogeneity of variance were visually inspected (normal probability plots, and fitted model versus observed values) and tested for using the Shapiro Wilk normality test and Levene's test ($W = 0.992$, $p = 0.074$; Levene's test with temperature treatment as grouping factor, $F_1 = 1.534$, $p = 0.216$). Significance of random effects was tested by model comparison. Wald Chi-square and p-values were computed using the 'car' package ([9]). These models were refitted by the maximum likelihood (ML) estimation method, which is suitable for mixed models in combination with a nested design.

BOX SA1: "Genotypic values"
Genotypic values refer to the average trait value of a given genotype in a common garden treatment. The measured value of a trait, or its phenotypic value, is typically conceptualized (e.g. [10] and [11]) as a sum of two components, one linked to the specific assemblage of segregating genes relevant to the phenotype in question (the genotypic value, G), and the other linked to all the non-genetic elements affecting the phenotype (environmental deviation, E).

As we assessed body size on different clonal lineages of different populations in a common garden setting in the laboratory (which controls for and thus randomizes any environmental deviations, so that interference from non-genetic factors can be excluded), the average phenotypic trait value of a given clone as quantified in our common garden experiment represents the genotypic value for the measured trait (here: body size) for that clone. We note that the difference between the measurements at 20 °C and 24 °C is induced by the change in temperature and thus quantifies phenotypic plasticity of the clones (note that given that we measure this phenotypic plasticity under controlled conditions we actually measure genotypic values for this phenotypic plasticity; our results indeed show that there is genotype-dependent phenotypic plasticity).

The relative contribution of genotypic trait variation (GTV), non-genetic intraspecific trait variation ($ITV_{PLASTICITY/OTHER}$) and interspecific trait turnover (SPT) to the change in cladoceran community body size

To disentangle the relative contribution of genotypic (GTV) and non-genetic trait variation (i.e. phenotypic plasticity or ontogenetic shifts; $\mathrm{ITV_{PLASTICITY/OTHER}}$) to the observed change in average community body size along the urbanization gradients, we modified the method of Lajoie & Vellend ([7]). The common garden experiment yielded genotypic trait values for 12 *D. magna* populations at 20 °C and 24 °C. Of the 10 communities containing *D. magna* in the community dataset, 7 were shared between both datasets. For the other three *D. magna* communities (PL3-yel, PL16-yel and Gent-CPB) genotypic trait values were estimated using the regression function from the common garden experiment (Fig. 3 main text). We estimated the expected average genotypic values for size at maturity using the regression equations relating genotypic values for size at maturity against percentage built-up area at 20 °C and 24 °C (see Table SA4 for values of the percentage built-up area for the three populations). For genotypic values for size at maturity at 20 °C we used the function

$$f_{20}(x) = 2.99958 - 0.12293 \log(x + 1),$$

and at 24°C we used the function

$$f_{24}(x) = 2.84065 - 0.06929 \log(x + 1),$$

with x the percentage built-up area.

To determine the contributions of genotypic and non-genetic trait variation along the urbanization gradient, we first rewrote the phenotypic trait value z_i for a species i as the sum of its genotypic trait value (GTV; z_i^G), its plasticity response to temperature ($\mathrm{ITV_{PLAST\text{-}T}}$; $z_i^T - z_i^G$) and its plasticity response to other environmental conditions present in the field or ontogenetic changes ($\mathrm{ITV_{OTHER}}$; $z_i - z_i^T$), i.e.

$$z_i = z_i^G + (z_i^T - z_i^G) + (z_i - z_i^T) \tag{SA1}$$

with z_i^G the genotypic trait value calculated as the average of the trait values in the 20 °C and 24 °C treatments, and z_i^T the estimated effect of plasticity in response to temperature (T), based on an expected relationship between percentage built-up area and temperature by translating the urbanization gradient to a temperature gradient. Based on data from temperature loggers in urban versus rural ponds we assumed that the average difference in summer temperature between the most urban and the most rural ponds is approximately 4 °C (as reported in Table SA3 and shown in Fig. SA2). We then assumed the most simple relationship between the degree of urbanization and temperature i.e. a linear relationship with %BA. Assuming this relationship we could derive expected values for temperature in the different ponds from which we obtained *D. magna* populations. In practice, because we did the experiment at 20 °C and 24 °C, the gradient in urbanization translated into a gradient between 20 °C and 24 °C. Our analysis does refer to summer conditions. We performed the following calculations:

$$a = \frac{max(\%BA) - x}{max(\%BA) - min(\%BA)},$$
$$z_{int} = (1 - a)z_{20} + az_{24},$$

with x the percentage built-up area of the *D. magna* population, $max(\%BA)$ (resp. $min(\%BA)$) the maximum (resp. minimum) value of percentage built-up area and z_{20} (resp. z_{24}) the genotypic value of size at maturity at 20 °C (resp. 24 °C). Substituting eqn (SA1) into the ITV model of Lajoie & Vellend ([7]) results for each community j consisting of s_j species in

$$\begin{aligned}\sum_{i=1}^{s_j} q_{ij}(z_{ij} - \bar{z}_i) = \sum_{i=1}^{s_j} q_{ij}(z_{ij}^G - \bar{z}_i^G) + \sum_{i=1}^{s_j} q_{ij}\big([z_{ij}^T - z_{ij}^G] - [\bar{z}_i^T - \bar{z}_i^G]\big) \\ + \sum_{i=1}^{s_j} q_{ij}\big([z_{ij} - z_{ij}^T] - [\bar{z}_i - \bar{z}_i^T]\big).\end{aligned} \tag{SA2}$$

To calculate the abundance-weighted average community body size for these 10 communities, we used the available genotypic trait values of *D. magna*, combined with the metacommunity-wide body sizes for all other species present in the local communities. As a result, in these analyses the effects of ITV and GTV only reflect variation in the focal species *D. magna*. To determine the contribution of genotypic trait variation, temperature-related phenotypic plasticity, and phenotypic variation due to other environmental conditions or demographic population structure along the urbanization gradient, a regression analysis was performed on each of the three terms in the right hand side of eqn (SA2). To determine which contributor (GTV, $\text{ITV}_{\text{PLAST-T}}$, $\text{ITV}_{\text{OTHER}}$ or SPT) has the largest relative importance a similar formula was used as in Lajoie & Vellend ([7]). Two communities identified as outliers were not included in this analysis; the results including these communities are presented in Supplementary Information B (Table SB5 and Figure SB8).

References

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Tables

Table SA1: List of all species and their observed average body size (mm) across all communities they were present in. The large species (>1 mm) are indicated in bold.

Table SA2: List of the 84 communities and the number of species present. The average number of species per pond across all communities is given in bold.

Table SA3: Subset of temperature data monitored in two rural (Meerdaal and Houwaert) and urban (Leuven and Mechelen) ponds, during 2013 and 2014. The maximum observed day temperatures (measured between 6.00am and 9.30pm) are given in bold for each pond. $T_{urb}(^{\circ}C)$ and $T_{rur}(^{\circ}C)$ give the according average temperature of the two urban and rural ponds (for the according dates of measurement), respectively. $\Delta T[^{\circ}C]_{urb-rur}$ gives the difference between the latter two (for the according dates of measurement). The average maximum day temperature for each pond during summer 2014 (July-August) and the warmest two-week period in July (7/17/14-7/31/14) are given in the last two rows of the table. $T_{urb}(^{\circ}C)$, $T_{rur}(^{\circ}C)$ and $\Delta T[^{\circ}C]_{urb-rur}$ are calculated in a similar way as described before.

Table SA4: Abbreviation and coordinates of the *D. magna* populations used for the quantitative genetic analysis (except for the last three populations) and the integrated analysis of genotypic, non-genetic intraspecific and species trait turnover. The fourth and the fifth column give the level of urbanization (assessed as percentage built-up area representing housing and other buildings only) at a 3200 meter radius around the sampling site, and the number of randomly isolated experimental lineages that were used to assess genotypic values of size at maturity. The number of multi-locus genotypes (MLGs, i.e. distinct genotypic lineages) identified using 27 microsatellite markers is given between brackets.

Table SA5: Regression model output of SPT+ITV, SPT and ITV, for each scale of urbanization (upper: complete dataset without one outlier (PL25-red); middle: small-species dominated subset without one outlier (PL26-yel); lower: large-species dominated subset without two outliers (PL25-red and TP-Blap1-riv). Significant results ($p < 0.05$) are shown in bold. p-values smaller than 0.1 are indicated with a dot. Ratio ITV/SPT is calculated as $\mathrm{SSR_{ITV}}/(\mathrm{SSR_{ITV}} + \mathrm{SSR_{SPT}})$ (*).

Table SA6: Regression model output of SPT+ITV, SPT, ITV, $\mathrm{ITV_{PLAST\text{-}T}}$, $\mathrm{ITV_{OTHER}}$ and GTV for the 8 communities in which *D. magna* is present at the urbanization scale of 3200 m. Two communities were excluded from the analysis (PL25-red and TP-Blap1-riv). Results with these two communities included can be found in Table SB5 and Figure SB8 (Supplementary Information B). Ratio ITV (third column) is calculated as $\mathrm{SSR_{ITV}}/(\mathrm{SSR_{ITV}}+\mathrm{SSR_{SPT}})$ and ratio GTV (fourth column) is calculated as $\mathrm{SSR_{GTV}}/(\mathrm{SSR_{GTV}}+\mathrm{SSR_{PLAST\text{-}T}}+\mathrm{SSR_{OTHER}}+\mathrm{SSR_{SPT}})$. Similar calculations are done for the $\mathrm{ITV_{PLAST\text{-}T}}$, $\mathrm{ITV_{OTHER}}$ and SPT component. Significant results ($p < 0.05$) are shown in bold. p-values smaller than 0.1 are indicated with a dot.

Table SA1:

Species	Average body size (mm)
Alona costata	0.3200
Alona gutata	0.2624
Alona quadrangularis	0.3520
Alona rectangula	0.2893
Alonella exisa	0.2912
Alonella exigua	0.2258
Bosmina longirostris	0.3279
Ceriodaphnia sp.	0.5370
Chydorus sphaericus	0.2652
Daphnia cucullata	0.4997
Daphnia longispina	0.7818
Daphnia magna	**1.8859**
Daphnia obtusa	**1.1437**
Daphnia pulex	**1.3736**
Diaphanosoma brachyurum	0.5120
Eurycercus lamellatus	**1.4507**
Graptolebris testudinaria	0.3796
Iliocryptus agilis	0.3680
Leydigia quadrangularis	0.5189
Pleuroxus aduncus	0.4100
Pleuroxus denticulatus	0.4341
Pleuroxus trigonellus	0.3392
Pleuroxus truncatus	0.4572
Polyphemus pediculus	0.6371
Scapholeberis kingi	0.4181
Scapholeberis mucronata	0.5057
Simocephalus sp.	0.9609

Table SA2:

Pond	Number of species	Pond	Number of species	Pond	Number of species
PL1-GRE	5	PL11-GRE	6	PL21-GRE	6
PL1-RED	4	PL11-RED	4	PL21-RED	4
PL1-YEL	2	PL11-YEL	6	PL21-YEL	6
PL2-GRE	3	PL12-GRE	2	PL22-GRE	3
PL2-RED	6	PL12-RED	3	PL22-RED	6
PL2-YEL	3	PL12-YEL	7	PL22-YEL	4
PL3-GRE	2	PL13-GRE	3	PL23-GRE	4
PL3-RED	3	PL13-RED	4	PL23-RED	4
PL3-YEL	7	PL13-YEL	1	PL23-YEL	4
PL4-GRE	NA	PL14-GRE	7	PL24-GRE	5
PL4-RED	4	PL14-RED	3	PL24-RED	6
PL4-YEL	3	PL14-YEL	8	PL24-YEL	2
PL5-GRE	2	PL15-GRE	5	PL25-GRE	4
PL5-RED	4	PL15-RED	6	PL25-RED	1
PL5-YEL	2	PL15-YEL	6	PL25-YEL	3
PL6-GRE	4	PL16-GRE	1	PL26-GRE	3
PL6-RED	4	PL16-RED	6	PL26-RED	4
PL6-YEL	5	PL16-YEL	5	PL26-YEL	1
PL7-GRE	4	PL17-GRE	4	PL27-GRE	3
PL7-RED	4	PL17-RED	3	PL27-RED	5
PL7-YEL	4	PL17-YEL	6	PL27-YEL	5
PL8-GRE	2	PL18-GRE	7	GENT-CPB	6
PL8-RED	2	PL18-RED	1	MECH-KT2	6
PL8-YEL	3	PL18-YEL	5	TP-BLAP1-RIV	6
PL9-GRE	3	PL19-GRE	3	**Average**	**4**
PL9-RED	4	PL19-RED	1		
PL9-YEL	6	PL19-YEL	5		
PL10-GRE	4	PL20-GRE	3		
PL10-RED	5	PL20-RED	4		
PL10-YEL	5	PL20-YEL	2		

Table SA3:

$T_{MAX}(^{\circ}C)$							
Date/Location	Leuven	Mechelen	Houwaert	Meerdaal	$T_{urb}(^{\circ}C)$	$T_{rur}(^{\circ}C)$	$\Delta T[^{\circ}C]_{urb-rur}$
6/9/2014	24.51	23.16	18.68	**21.08**	23.16	19.88	3.28
7/20/14	25.74	25.48	**20.41**	20.13	25.61	20.27	5.43
7/19/14	**26.26**	26.11	20.13	19.84	26.19	19.99	6.2
7/23/14	25.43	**26.57**	19.96	20.22	26.00	20.09	5.91
$T_{MAX,average}(^{\circ}C)$							
Summer	20.90	21.95	17.51	21.43	21.43	17.4	**4.03**
7/17/14-7/31/14	23.53	23.59	19.42	19.46	23.56	19.44	4.12

Table SA4:

	Pond location		BA (%)	Experimental lineages
Population	*Latitude (N)*	*Longitude (E)*	3200 m	*(n° of MLGs)*
Laps	51.28253	3.355467	0.637	6(5)
Damm	51.26207	3.276039	1.125	6(6)
ZwMe	50.82274	4.653691	2.017	6(6)
MidL	50.98233	5.317858	3.721	6(5)
Gera	50.7842	3.915978	4.382	6(3)
BuSN	51.17808	4.161051	12.479	6(4)
OudM	50.86328	4.723935	13.188	6(5)
Mech	51.02402	4.484039	14.125	6(2)
FaSN	51.15623	4.159557	14.995	6(5)
BppK	50.81624	3.271563	15.356	6(6)
GenC	51.0389	3.723744	24.242	6(6)
GenM	51.04251	3.731611	25.736	6(5)
PL3-yel	50.843175	4.860750	2.627	/
PL16-yel	50.797084	4.549610	4.118	/
Gent-CPB	51.036658	3.718006	22.916	/

Table SA5:

	SPT+ITV		SPT			ITV			Ratio ITV/SPT
Urbanization	*Slope*	*p-value*	*SSR/SST*	*Slope*	*p-value*	*SSR/SST*	*Slope*	*p-value*	*
Total set of communities									
50 m	**-0.077**	**< 0.001**	**0.1509**	**-0.078**	**< 0.001**	0.0004	0.004	0.709	0.0030
100 m	**-0.059**	**0.006**	**0.0991**	**-0.061**	**0.002**	0.0014	0.007	0.512	0.0137
200 m	-0.041	0.074 ·	**0.0527**	**-0.048**	**0.025**	0.0048	0.014	0.222	0.0827
400 m	-0.037	0.145	0.0351	-0.043	0.069 ·	0.0044	0.015	0.240	0.1111
800 m	-0.018	0.495	0.0090	-0.023	0.362	0.0028	0.013	0.346	0.2400
1600 m	-0.020	0.533	0.0069	-0.023	0.424	0.0017	0.012	0.464	0.1990
3200 m	-0.024	0.557	0.0063	-0.029	0.446	0.0009	0.011	0.592	0.1276
Small-species dominated communities									
Urbanization	*Slope*	*p-value*	*SSR/SST*	*Slope*	*p-value*	*SSR/SST*	*Slope*	*p-value*	*
50 m	**-0.068**	**< 0.001**	**0.2334**	**-0.058**	**< 0.001**	0.0173	-0.016	0.052 ·	0.0691
100 m	**-0.057**	**< 0.001**	**0.1599**	**-0.048**	**< 0.001**	0.0148	-0.015	0.074 ·	0.0848
200 m	**-0.051**	**0.007**	**0.1176**	**-0.045**	**0.004**	0.0042	-0.009	0.349	0.0342
400 m	-0.040	0.064 ·	0.0431	-0.031	0.091 ·	0.0087	-0.014	0.175	0.1672
800 m	-0.021	0.326	0.0075	-0.013	0.488	0.0079	-0.013	0.195	0.5135
1600 m	-0.018	0.469	0.0025	-0.009	0.688	0.0080	-0.015	0.193	0.7600
3200 m	-0.014	0.666	0.0021	-0.010	0.715	0.0011	-0.007	0.639	0.3350
Large-species dominated communities									
Urbanization	*Slope*	*p-value*	*SSR/SST*	*Slope*	*p-value*	*SSR/SST*	*Slope*	*p-value*	*
50 m	-0.003	0.929	0.0105	-0.016	0.530	0.0301	0.028	0.262	0.7416
100 m	0.023	0.402	0.0014	0.006	0.822	0.0595	0.036	0.111	0.9777
200 m	0.042	0.115	0.0232	0.023	0.348	0.0786	0.042	0.065 ·	0.7720
400 m	**0.057**	**0.046**	0.0431	0.034	0.198	**0.1063**	**0.053**	**0.030**	0.7114
800 m	**0.066**	**0.034**	0.0548	0.041	0.145	**0.1008**	**0.056**	**0.035**	0.6477
1600 m	**0.085**	**0.020**	0.0812	0.059	0.074 ·	0.0848	0.060	0.055 ·	0.5107
3200 m	**0.110**	**0.024**	0.0944	0.085	0.053 ·	0.0468	0.060	0.159	0.3314

Table SA6:

	Slope	*p-value*	*SSR/SST*	*Ratio ITV*	*Ratio GTV*
ITV	0.154	0.056 ·	0.3243	0.9578	/
GTV	-0.043	0.137	0.0255	/	0.0455
$ITV_{PLAST\text{-}T}$	0.001	0.853	1.28e-05	/	2.30e-05
ITV_{OTHER}	**0.195**	**0.047**	**0.5194**	/	0.9289
SPT	0.032	0.760	0.0143	0.0422	0.0256
SPT+ITV	0.089	0.423	1		

Figures

Figure SA1: Sampling locations of all 84 zooplankton communities in Flanders. Levels of urbanization (< 5 %, green; 5 - 10 %, yellow; > 10 % red) are given for both the 50 m (inner point) and 3200 m radii (outer circle). Cities depicted have a minimum of 680,000 inhabitants.

Figure SA2: Visualization of average day temperature ($°C$, measured between 6.00am and 9.30pm) monitored in two rural (dashed lines) and two urban (solid lines) ponds during the summer (July-August) of 2014. The average difference in maximum day temperature between urban and rural ponds was approximately 4.03 °C during this period.

Figure SA3: Visualization of the outliers that were removed for the analyses performed in the main text. Left column plots show the relationship between body size variation and percentage built-up area (%BA + 1, plotted on a log-scale) among the (a) 84 communities, (c) the subset of communities dominated by small species and (e) the subset of communities dominated by large species, where average community body size is calculated using the local trait values of the species (filled triangles) or using the metacommunity-wide species trait values (unfilled triangles). Right column plots show the difference in average community body size when using local versus metacommunity-wide species trait values for the (b) 84 communities, (d) the subset of communities dominated by small species and (f) the subset of communities dominated by large species. In the right corner of each graph we denoted the spatial scale of urbanizationn. Outliers are colored in grey and indicated with a circle.

Figure SA4: Relationships between abundance-weighted average community body size and percentage built-up area (%BA + 1) as quantified at different spatial scales (100, 200, 400, 800 and 1600 meter; plotted on a log-scale) for the total set of 83 communities (a, d, g, j, m), the subset of communities dominated by small species ($n = 49$; b, e, h, k, n), and the subset of communities dominated by large species ($n = 32$; c, f, i, l, o), where average community body size is calculated using the local trait values of the species (filled symbols) and using the metacommunity-wide species trait values (unfilled symbols). Significant relationships ($p < 0.05$) between average community body size and percentage built-up area (%BA + 1), when using local or metacommunity-wide species trait values, are represented by a solid or dashed line, respectively. For $p < 0.1$ regression lines are given in grey. p-values can be found in Table SA5.

Figure SA5: Reaction norms for size at maturity (mm; ± 1 SE) of the different clones isolated from the 12 *Daphnia magna* populations as quantified in the common garden experiment, plotted along the percentage built-up area of the pond from which the populations were isolated. Empty symbols refer to size at maturity at 20 °C, filled symbols to size at maturity at 24 °C.

Figure SA6: The difference in measurements of body size values when both adults and juveniles are taken into account and when only adults are taken into account plotted against percentage built-up area (quantified at a scale of 3200 m). Values larger than zero imply that body size values that only take adults into account are larger than when adults and juveniles are taken into account. One influential point (TP-Blap1-riv) was removed from the analysis and is colored in grey and indicated with a circle. The regression has a slope of -0.048 and is significant ($p = 0.032$).

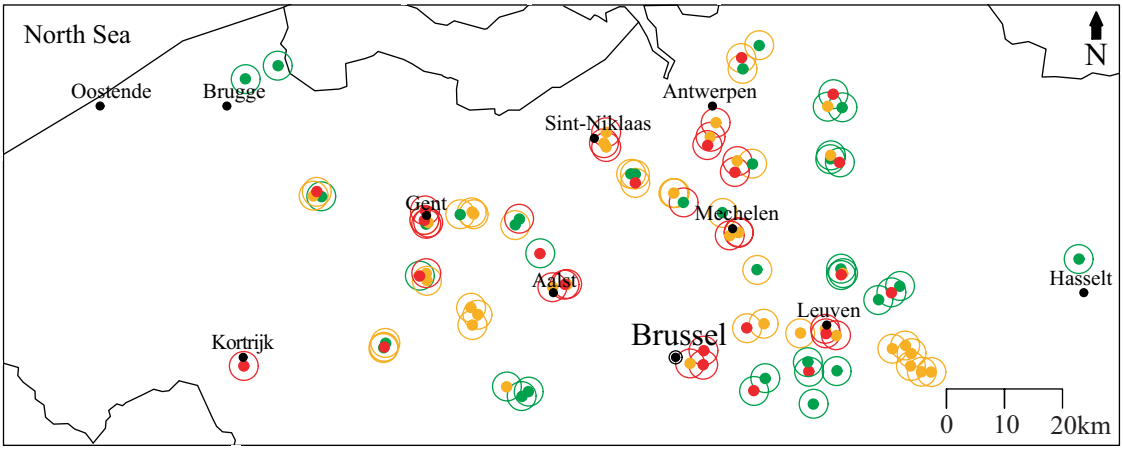

Figure SA1:

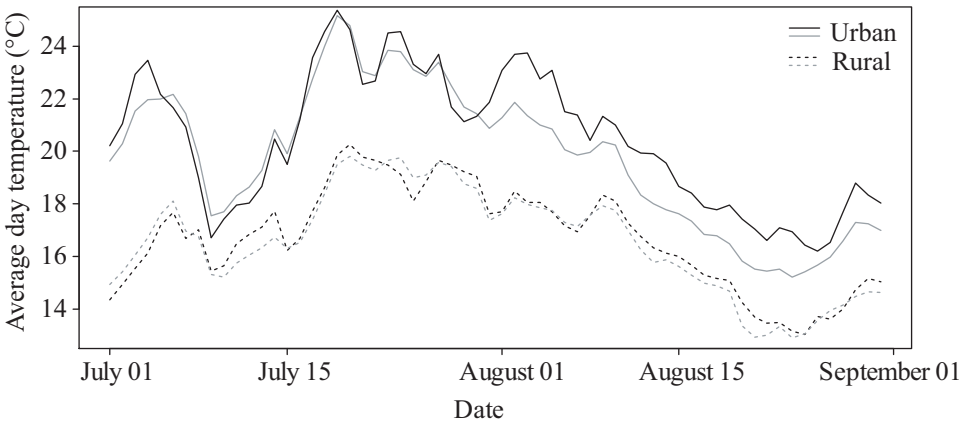

Figure SA2:

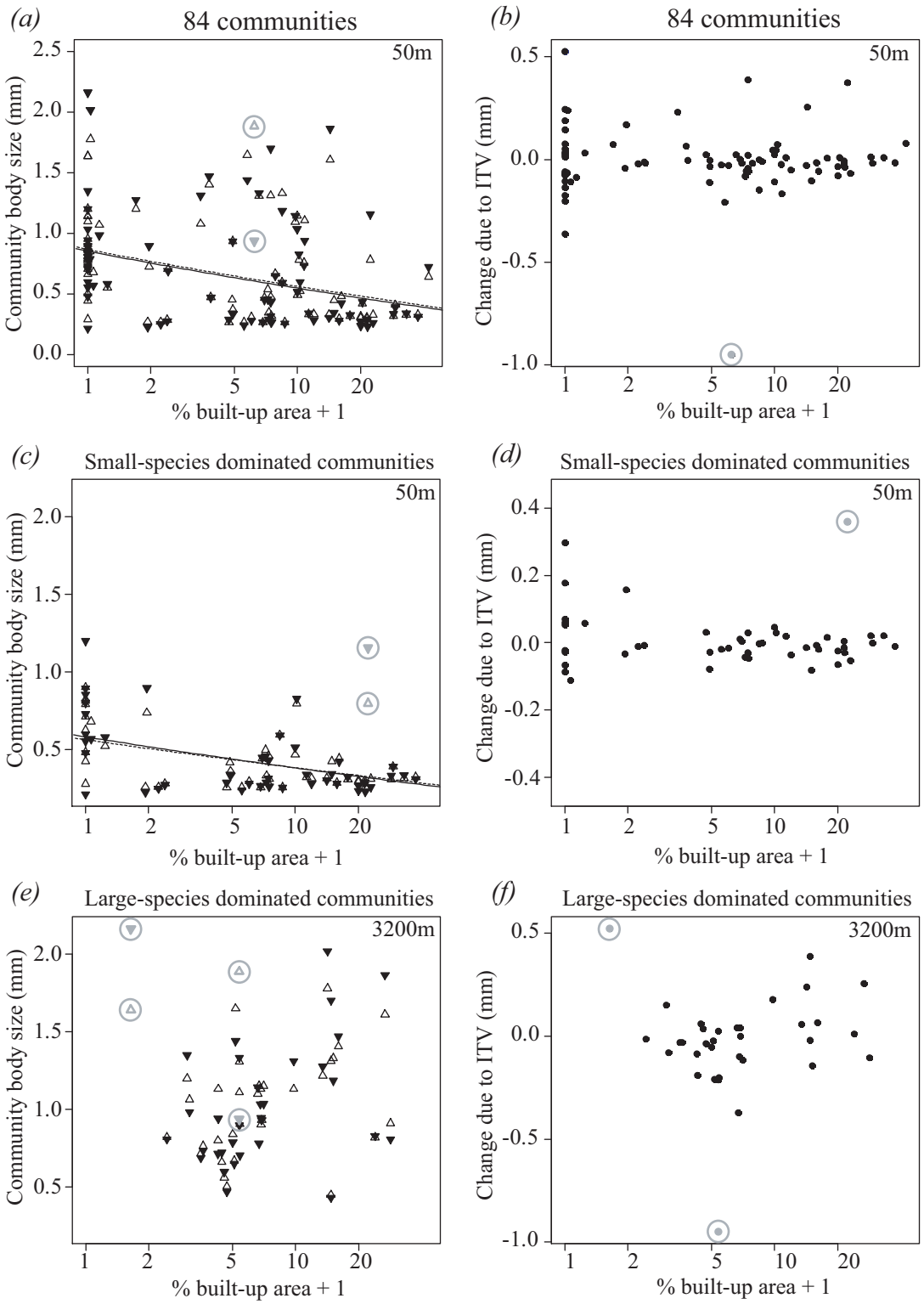
(a)
84 communities
50m
Community body size (mm)
% built-up area + 1
(b)
84 communities
50m
Change due to ITV (mm)
% built-up area + 1
(c)
Small-species dominated communities
50m
Community body size (mm)
% built-up area + 1
(d)
Small-species dominated communities
50m
Change due to ITV (mm)
% built-up area + 1
(e)
Large-species dominated communities
3200m
Community body size (mm)
% built-up area + 1
(f)
Large-species dominated communities
3200m
Change due to ITV (mm)
% built-up area + 1

Figure SA3:

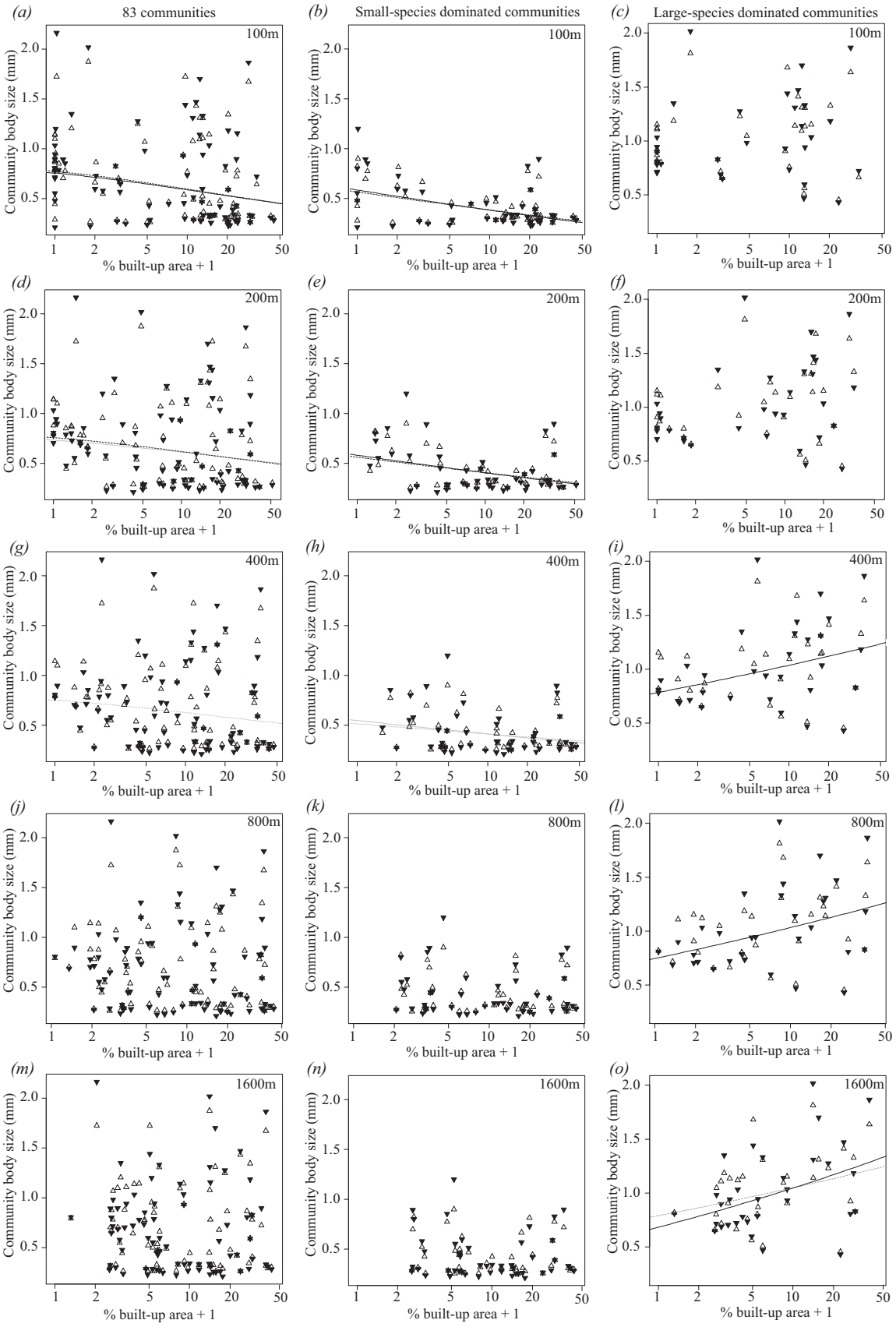
(a)
83 communities
(b)
Small-species dominated communities
(c)
Large-species dominated communities
100m
200m
400m
800m
1600m
Community body size (mm)
% built-up area + 1
0.5
1.0
1.5
2.0
1
2
5
10
20
50

Figure SA4:

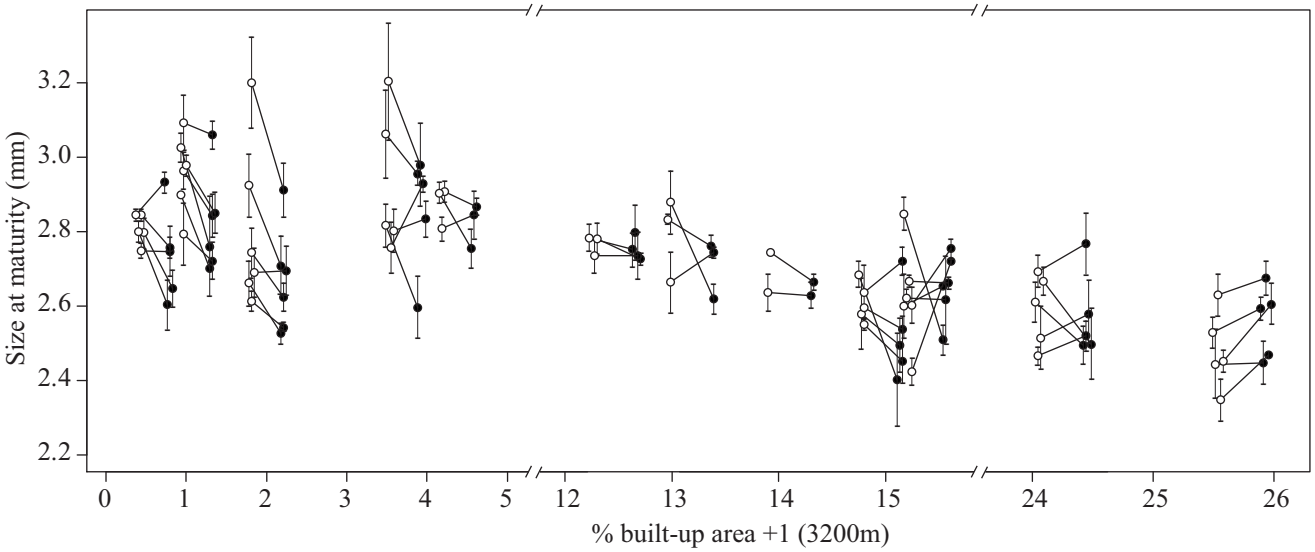

Figure SA5:

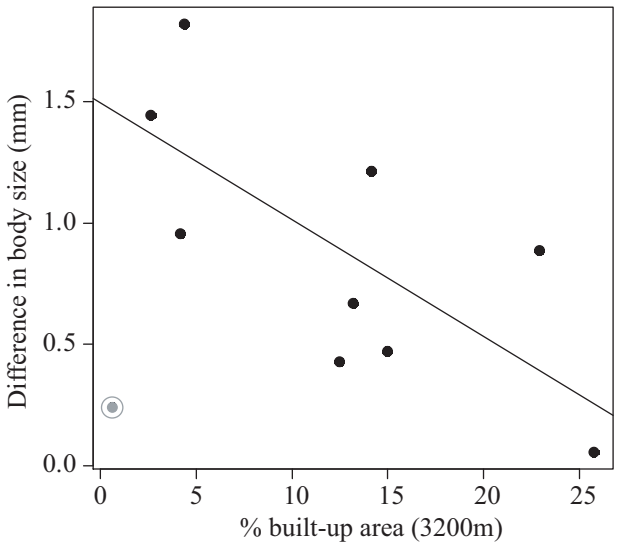

Figure SA6: