Supplementary Information A "Urbanization drives genetic differentiation in physiology and structures the evolution of pace-of-life syndromes in the water flea *Daphnia magna*"

Brans Kristien I, Stoks Robby & De Meester Luc

A. FIGURES AND TABLES

Figures

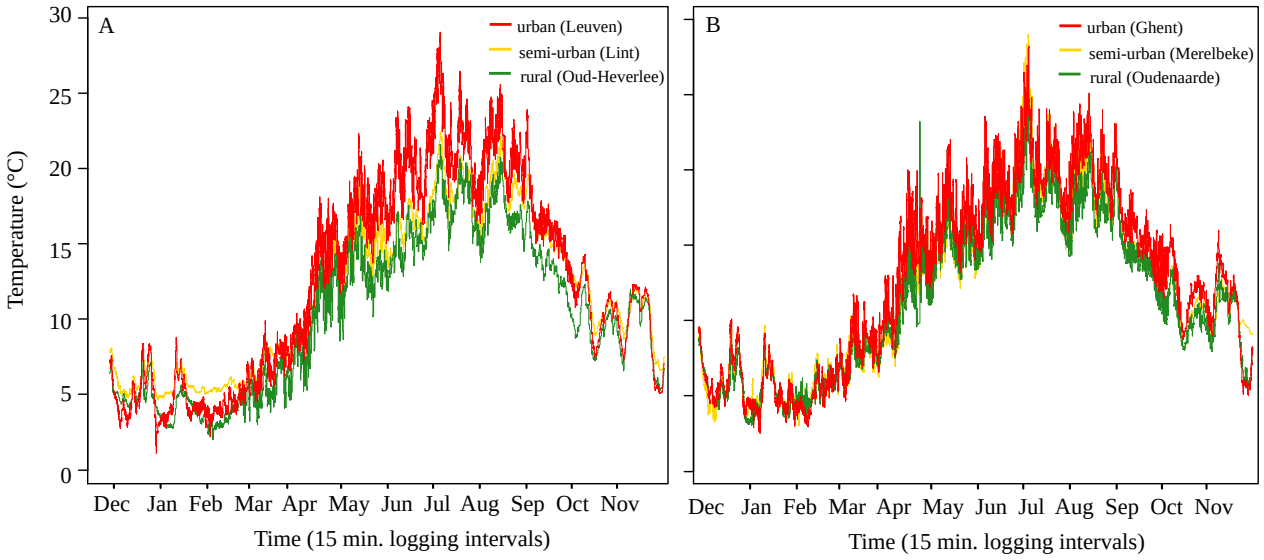

Figure S1. Temperature profiles (°C, measured at 15 min. intervals; period November 2014- November 2015) for an urban (red), semi-urban (yellow), and rural (green) pond in the regions of (A) Leuven and (B) Gent in Flanders, Belgium, in the eastern and western part of the region of Flanders, respectively (adapted from [1]).

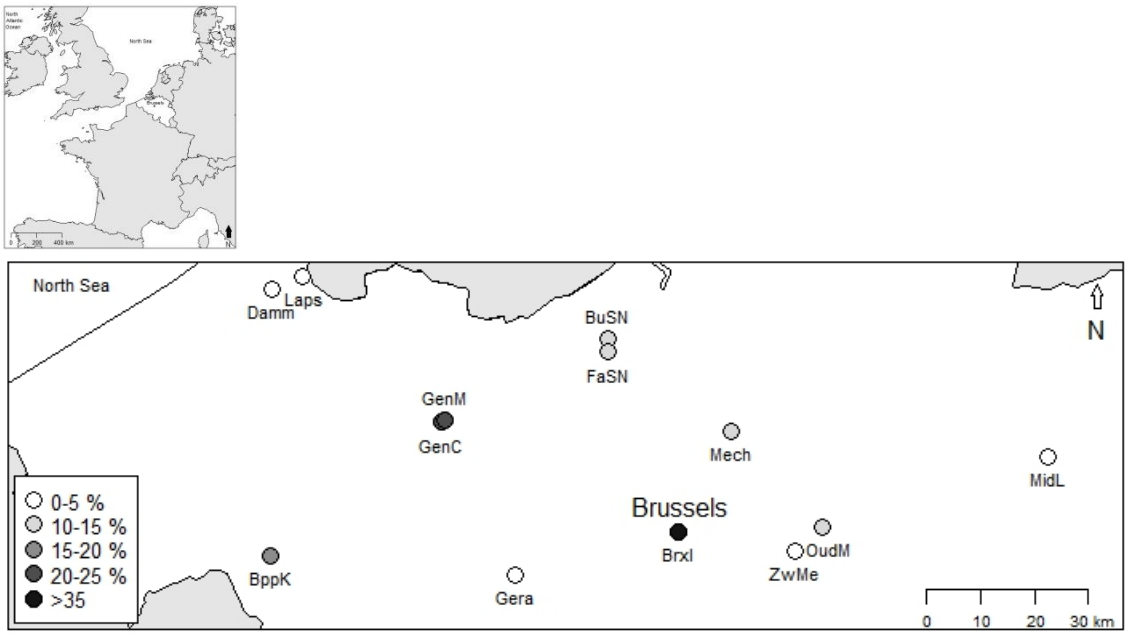

Figure S2. Sampling locations of the *D. magna* study populations in Flanders. Urbanization classes are given according to the percentage built-up area in a radius of 3200 m around each pond (< 5% BA: rural, *n* = 5; > 10% BA: urban, *n* = 8). Inset: Wider region of Europe, depicting Belgium. Adapted from [2,3].

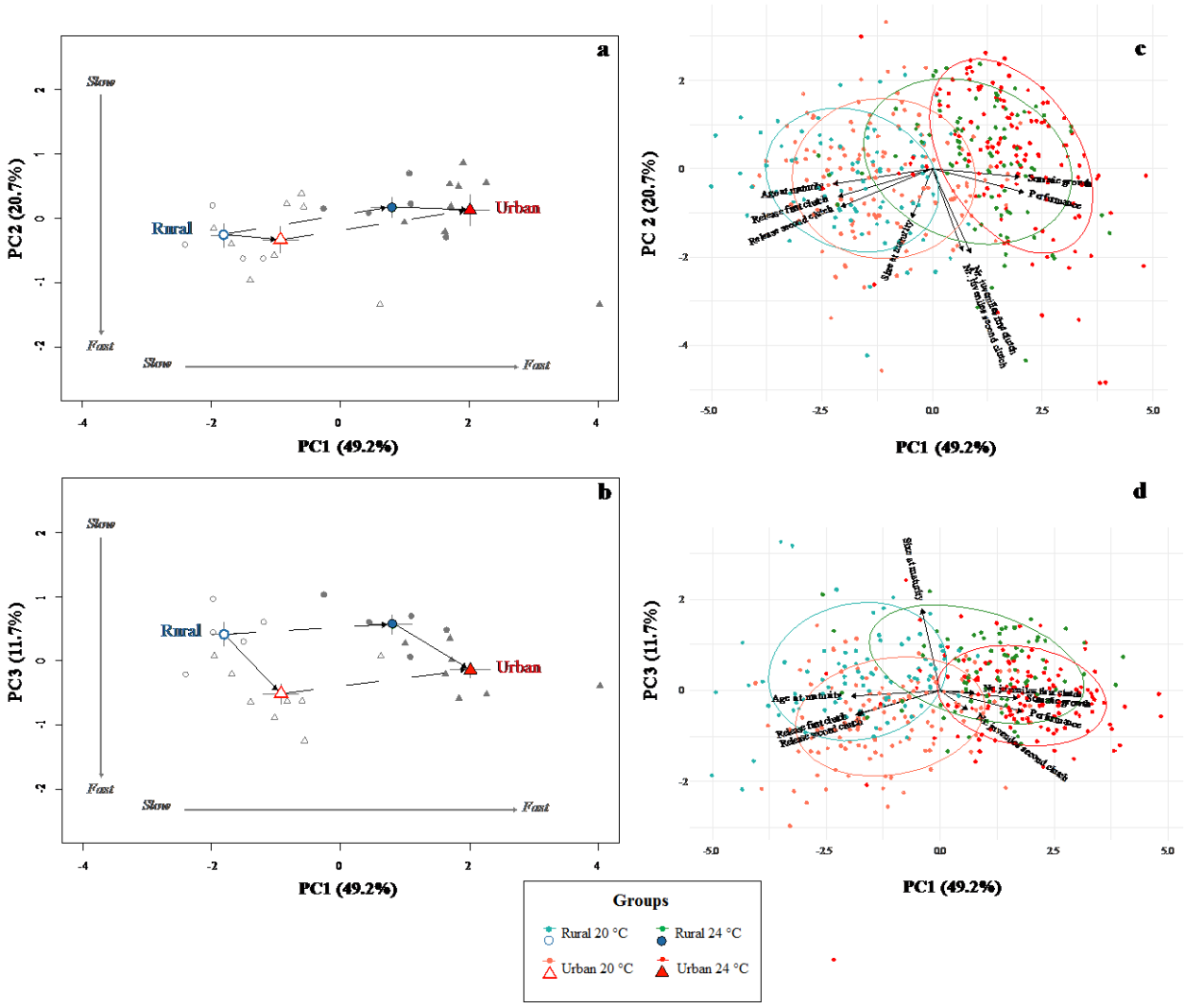

Figure S3. Multivariate life history results. Principal Component Analysis plots showing: (a,b) phenotypic trajectories (multivariate response to experimental warming for eight morphological and life history traits; dashed lines) and genotypic trajectories (multivariate differentiation between rural and urban animals; full lines) for rural (blue circles) and urban (red triangles) populations of the water flea *Daphnia magna* reared at 20 (open symbols) and 24 °C (full symbols); population means are depicted in grey shades (white: 20 °C, gray: 24 °C), average 'urban' and 'rural' centroids are depicted in the according color (rural: blue; urban: red); (c,d) PCA plots showing all observations (n = 440) grouped according to the urbanization x temperature combinations (rural-20 °C: blue, rural-24 °C: green, urban-20 °C: orange, urban-24 °C, red). Patterns are given for PC1 and PC2 (a,c) and PC1 and PC3 (b,d). An indicative 'pace-of-life' axis along all PC axes is given in plots a and b. Adapted from [4].

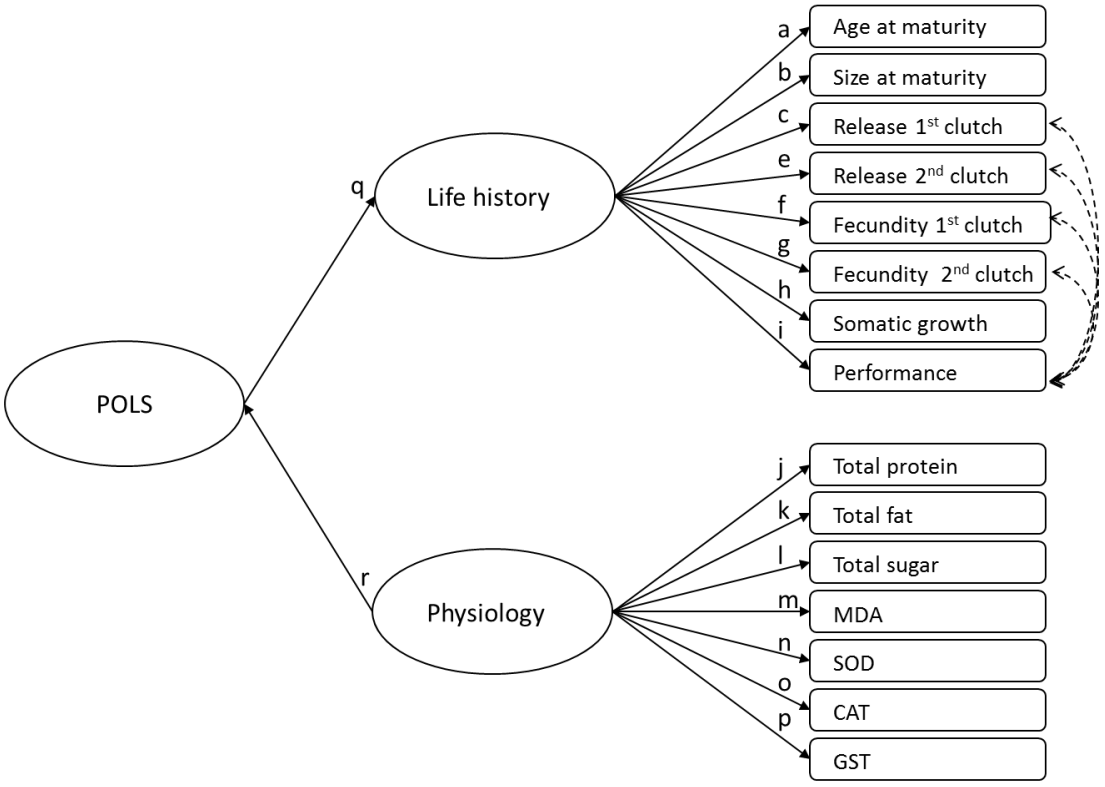

Figure S4. Latent variable structural equation model. A priori path model between life history traits and physiology traits, potentially shaped by a life-history syndrome structure (paths a-i),a physiology syndrome sturcure (paths j-p), and an overarching syndrome structure (paths q and r) according to the pace-of-life Syndrome Hypothesis [5]. “Syndromes” underlying the possible trait covariation of measured variables (boxes) at each level of biological organization are modelled as ‘latent’, non-measured, variables (ovals). Dashed arrows are correlated errors, indicating residual covariation not related to the syndrome structure or path model.

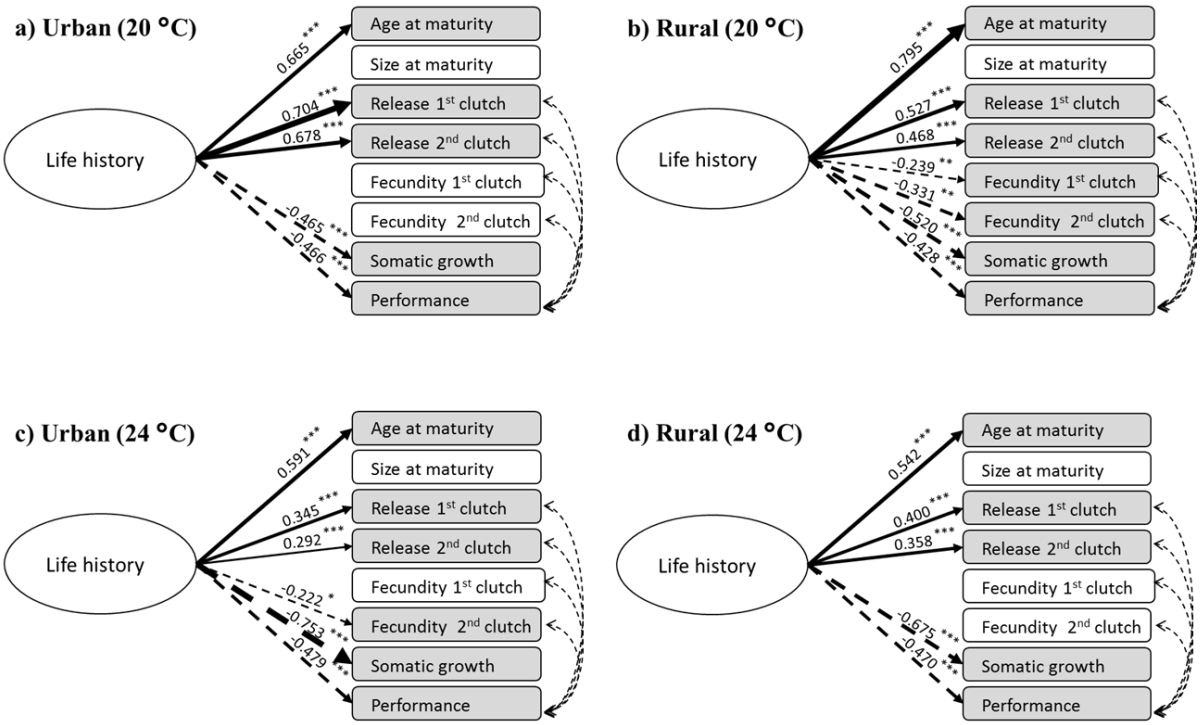

Figure. S5. Structural equation models life history syndrome. Path models showing the covariation pattern of measured life history traits (boxes) within an overarching life history syndrome (modeled as latent variable, ovals) in urban (a,c) and rural (b,d) genotype sets of *Daphnia magna* when reared at 20 °C (a,b) and 24 °C (c,d). The best model supporting the underlying syndrome structure was characterized by factor loadings free across all temperature x urbanization groups (Model IV, Table S3). Only paths significantly contributing to syndrome structure (i.e. significantly different from 0) are depicted (associated traits are in grey colored boxes), and accompanied with path coefficients. Significance levels of path coefficients are depicted as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Path coefficients indicate the change (in SD units) of traits predicted to occur based on a 1 SD change in the underlying syndrome structure. Arrow thickness (dashed arrows: negative correlations, full arrows: positive correlations) is proportional to the strength of the path loading. Dashed double-headed arrows indicate correlated errors.

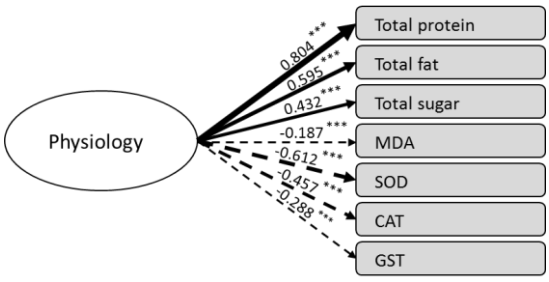

Figure S6. Structural equation model physiology syndrome. Path model showing the covariation pattern of measured stress physiology traits (boxes) within an overarching physiology syndrome (modeled as latent variable, ovals). The best model supporting the underlying syndrome structure was characterized by factor loadings constraint across all temperature x urbanization groups (Model I, Table S3). Only paths significantly contributing to syndrome structure (i.e. significantly different from 0) are depicted (associated traits are in grey colored boxes), and accompanied with path coefficients. Significance levels of path coefficients are depicted as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Path coefficients indicate the change (in SD units) of traits predicted to occur based on a 1 SD change in the underlying syndrome structure. Arrow thickness (dashed arrows: negative correlations, full arrows: positive correlations) is proportional to the strength of the path loading.

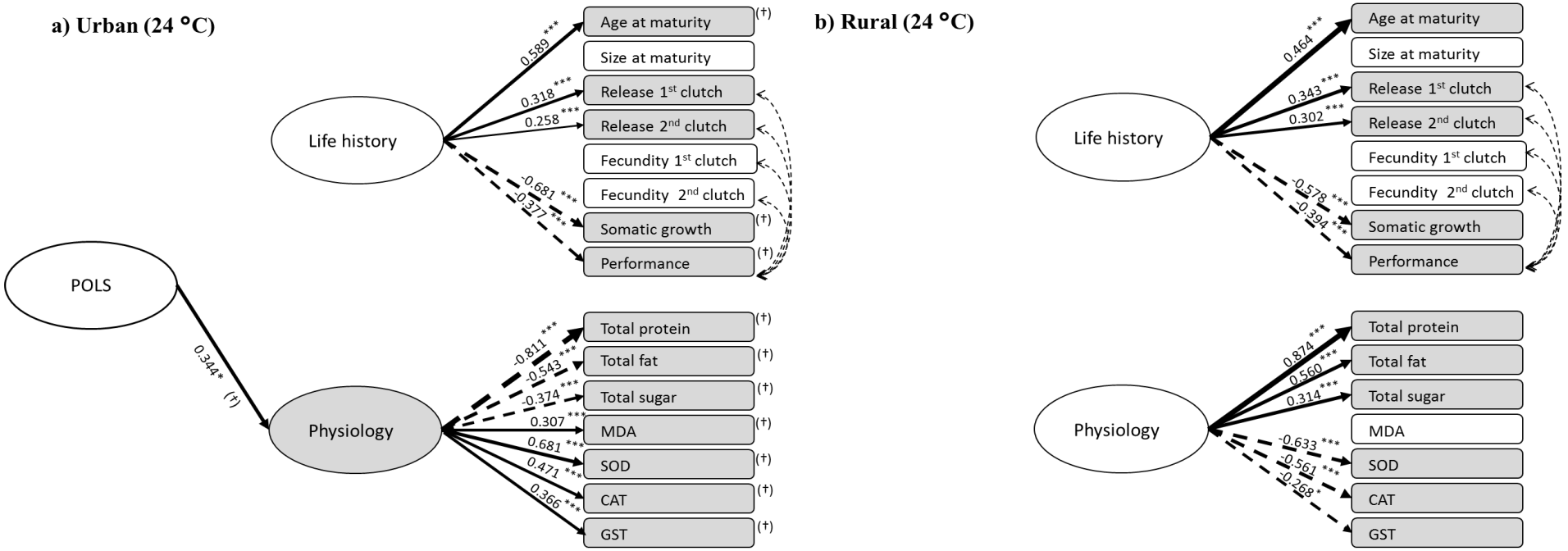

Figure. S7. Structural equation models. Path models showing the covariation pattern of life history and physiological traits (i.e. measured traits, boxes) within a life history, physiology, and overarching pace-of-life syndrome (POLS) (all modeled as latent variable, ovals) in a) urban and b) rural genotype sets of *Daphnia magna* when reared at 24 °C. Only paths significantly contributing to syndrome structure (i.e. significantly different from 0) are depicted (associated traits are in grey colored boxes), and accompanied with path coefficients. Significance levels of path coefficients are depicted as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Path coefficients indicate the change (in SD units) of traits predicted to occur based on a 1 SD change in the underlying syndrome structure. Arrow thickness (dashed arrows: negative correlations, full arrows: positive correlations) is proportional to the strength of the path loading. Path loadings significantly differing between urban and rural genotype sets (either because of their significant contribution to a syndrome in one group, but not in the other, or because of a significant difference in direction or size of path loadings that significantly contribute to syndrome structures in both groups) are indicated with † ($p < 0.05$ after Bonferroni correction). Dashed double-headed arrows indicate correlated errors.

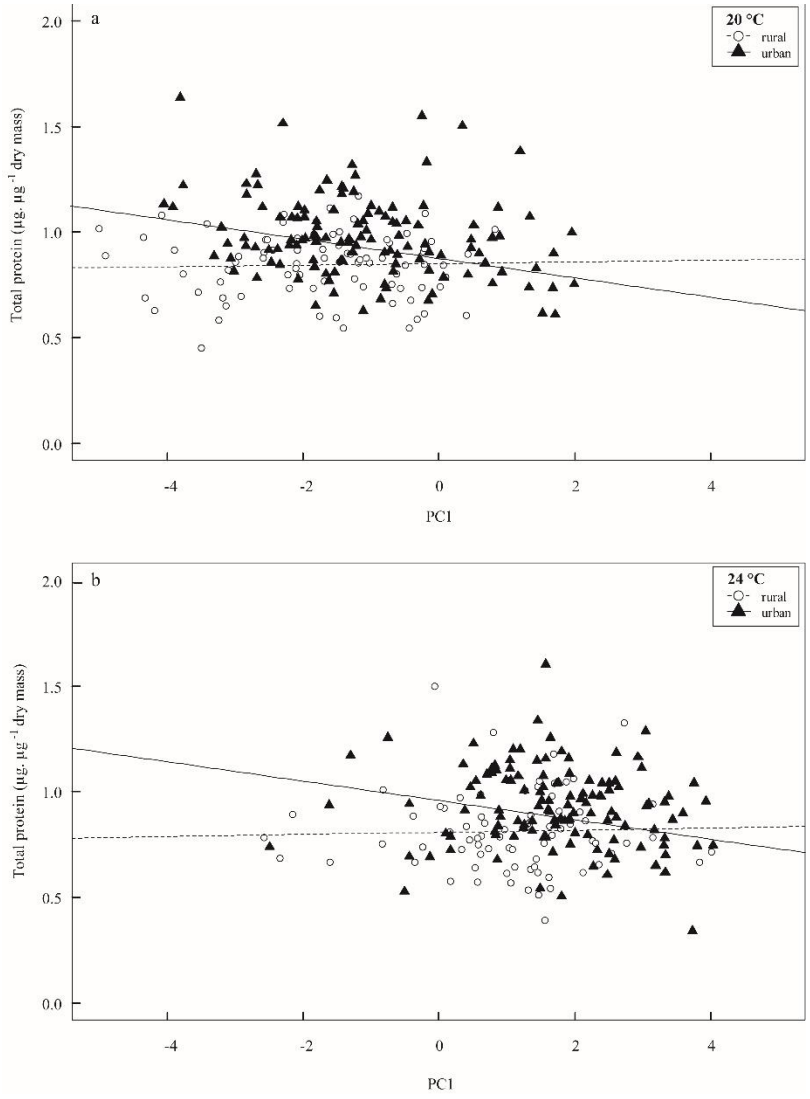

Figure. S8. Total protein content regressed against pace-of-life (life history). Total protein content plotted against PC1 scores from a multivariate analyses including all measured life history traits in urban (blak triangels) and rural (white circles) *D. magna* genotypes. Regression lines are computed from the statistical output from a linear mixed-effect model, including the effects of PC1, urbanization, temperature, and all possible interactions between the latter on total protein content. A significant PC1 x urbanization interaction effect ($p = 0.008$) was observed, indicating slopes differed in urban (negative correlation) and rural (no correlation) genotype sets. For the sake of clarity, we here plotted data obtained from both temperature treatments (20 °C and 24 °C) separately.

Tables

Table S1. *Daphnia magna* populations. Locations of the *D. magna* study populations including the percentage built-up area (BA) in a radius of 3200 m around each pond, the according urbanization categorization, and the number of experimental lines and clones (i.e. multi-locus genotypes, MLGs) used in the experiment. Adapted from [3].

Population		**Location**		**Urbanization**		**Exp. lines**
Pond	Code	Latitude (N)	Longitude (E)	%BA	Cat.	(MLGs)
Brussel	*Brxl*	50.855464	4.347334	40.440	urban	4(4)
Damme	*Damm*	51.26207	3.276039	1.126	rural	6(6)
Gent (Citadelpark)	*GenC*	51.03890	3.723744	24.242	urban	6(6)
Gent (Muinkpark)	*GenM*	51.04251	3.731611	25.736	urban	6(5)
Geraardsbergen	*Gera*	50.78420	3.915978	4.382	rural	6(3)
Kortrijk (Blauwe Poort)	*BppK*	50.81624	3.271563	15.356	urban	6(6)
Lapscheure	*Laps*	51.28253	3.355467	0.637	rural	6(5)
Leuven (Oude-Meren)	*OudM*	50.86328	4.723935	13.188	urban	6(5)
Mechelen (Kruidtuin)	*Mech*	51.02402	4.484039	14.125	urban	6(2)
Meerdaal (Zoete Waters)	*ZwMe*	50.82274	4.653691	2.017	rural	6(6)
Midden-Limburg (BKN1)	*MidL*	50.98233	5.317858	3.721	rural	6(5)
Sint-Niklaas (Bufferbekken)	*BuSN*	51.17808	4.161051	12.479	urban	6(4)
Sint-Niklaas (Fabiola Park)	*FaSN*	51.15623	4.159557	14.995	urban	6(5)

Table S2. Multi-group structural equation modeling (SEM). Alternative path models to test for i) overall covariation in life history and physiological traits according to a life history, physiology, and overarching pace-of-life syndrome, ii) differences in model structure across urbanization level and/or temperature treatment for the life history, physiology, and overarching pace-of-life syndrome. Each model is a multi-group SEM with all combinations of urbanization x temperature treatment as grouping structure. Each alternative SEM is characterized by a different set of constrained factor loadings (all, temperature, urbanization level, none; equal factor loadings are indicated by an identical character). For more information on the interpretation on model structures we refer to Supplementary Information B: Material and Methods.

		Urban		Rural	
Model		20 °C	24 °C	20 °C	24 °C
I	All loadings constrained equal across treatments and urbanization level	A	A	A	A
II	Loadings constrained across temperature treatment (*free across urbanization level)*	A	A	B	B
III	Loadings constrained across urbanization level *(free across temperature treatment)*	A	B	A	B
IV	All loadings free	A	B	C	D

Table S3. Multi-group SEM results. Four models of covariation of life history and physiological traits along separate life history and physiological syndromes (A, B) and an overarching pace-of-life syndrome (C) were evaluated with factor loadings (I) constrained across both urbanization level and temperature treatment groups, (II) constrained across temperatures (free across urbanization levels), (III) constrained across urbanization levels (free across temperatures), (IV) free across all groups. Model AICs are given together with ΔAIC (based on the best fitting model's AIC score), together with model weights (*W*) and evidence ratios (E.R.). The optimal model for each syndrome structure is given in bold. Competing models (ΔAIC < 2) are given in italics.

	Model	Factor levels constrained across	AIC	ΔAIC	*W*	E.R.
A) Life history SEMs	I	Temp. x Urb.	5739.60	134.38	0.00	$7.35\ E^{+39}$
	II	Temperature	5744.18	188.168	0.00	$7.25E^{+40}$
	III	Urbanization	5579.97	23.96	0.00	159452.28
	IV	**All free**	**5556.01**	**0**	**0.99**	**1**
B) Physiological SEMs	***I***	**Temp. x Urb.**	***7302.31***	***0***	***0.59***	***1***
	II	Temperature	*7303.99*	*1.68*	*0.25*	*2.32*
	III	Urbanization	7304.99	2.67	0.15	3.81
	IV	All free	7312.18	9.86	0.004	139
C) Full POLS SEMs	I	Temp. x Urb.	12602.85	131.7	0.00	$4.09E^{+28}$
	II	Temperature	12616.73	145.64	0.00	$4.22E^{+31}$
	III	Urbanization	12477.47	6.38	0.04	24.29
	IV	**All free**	**12471.09**	**0**	**1**	**1**

References

1. Brans KI, Engelen J, Souffreau C & De Meester L. 2018 Urban hot-tubs: Local urbanization has profound effects on average and extreme temperatures in ponds. *Landsc. Urban Plan.* **176**, 22-29. (doi:10.1016/j.landurbplan.2018.03.013)

2. Brans KI, Govaert L, Engelen J, Gianuca AT, Souffreau C & De Meester L. 2017a Eco-evolutionary dynamics in urbanized landscapes: Evolution, species sorting and the change in zooplankton body size along urbanization gradients. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **372** (1712). (doi:10.1098/rstb.2016.0030)

3. Brans KI, Jansen M, Vanoverbeke J, Tüzün N, Stoks R & De Meester L. 2017b The heat is on: Genetic adaptation to urbanization mediated by thermal tolerance and body size. *Glob.Change. Biol*. **23**, 5218-5227. (doi:10.1111/gcb.13784)

4. Brans KI & De Meester, L. 2018 (in press). City life on fast lanes: urbanization induces an evolutionary shift towards a faster life style in the water flea *Daphnia*. *Funct. Ecol.* (doi:10.1111/1365-2435.13184)

5. Réale D, Garant D, Humphries MM, Bergeron P, Careau V & Montiglio P-O. 2010 Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **365**, 4051-4063. (doi:10.1098/rstb.2010.0208)