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1 **Cryptic eco-evolutionary feedback in the city: urban evolution of prey dampens the effect of**  
2 **urban evolution of the predator**

3

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26 **ABSTRACT**

- 27 1. Most research on eco-evolutionary feedbacks focuses on ecological consequences of  
28 evolution in a single species. This ignores the fact that evolution in response to a shared  
29 environmental factor in multiple species involved in interactions could alter the net  
30 cumulative effect of evolution on ecology.
- 31 2. We empirically tested whether urbanization-driven evolution in a predator (nymphs of the  
32 damselfly *Ischnura elegans*) and its prey (the water flea *Daphnia magna*) jointly shape the  
33 outcome of predation under simulated heatwaves. Both interactors show genetic trait  
34 adaptation to urbanization, particularly to higher temperatures.
- 35 3. We cross-exposed common-garden reared damselflies and *Daphnia* from replicated urban and  
36 rural populations, and quantified predation rates and functional response traits.
- 37 4. Urban damselfly nymphs showed higher encounter and predation rates than rural damselflies  
38 when exposed to rural prey, but this difference disappeared when they preyed on urban  
39 *Daphnia*. This represents a case of a cryptic evo-to-eco feedback, where the evolution of one  
40 species dampens the effects of the evolution of another species on their interaction strength.  
41 The effects of evolution of each single species were strong: the scenario in which only the  
42 predator or prey was adapted to urbanization resulted in a ca. 250% increase in encounter rate  
43 and a ca. 25% increase in predation rate, compared to the rural predator - rural prey  
44 combination.
- 45 5. Our results provide unique evidence for eco-evolutionary feedbacks in cities, and underscore  
46 the importance of a multi-species approach in eco-evolutionary dynamics research.

47

48 **Key words:** cryptic urban eco-evolutionary feedbacks, *Daphnia*, heatwaves, *Ischnura*, predator-prey  
49 interactions, thermal adaptation.

50

51 **ABSTRACT (DUTCH)**

- 52 1. Onderzoek naar eco-evolutionaire terugkoppelingen focust vaak op de ecologische gevolgen  
53 van evolutie in één soort. Bijgevolg negeert men de mogelijkheid dat evolutionaire  
54 veranderingen van meerdere interagerende soorten als respons op een gedeelde  
55 omgevingsverandering met elkaar kunnen interfereren en zo het netto effect van evolutie op  
56 ecologische processen kan veranderen.
- 57 2. We testten empirisch of door verstedelijking gedreven evolutie in een predator (larven van de  
58 waterjuffer *Ischnura elegans*) en zijn prooi (de watervlo *Daphnia magna*) de uitkomst van  
59 predatie onder gesimuleerde hittegolven beïnvloedt. Beide interactoren vertonen genetische  
60 adaptatie aan de stadsomgeving, meer specifiek de daar voorkomende hogere temperaturen.
- 61 3. We stelden waterjuffers en watervlooien, afkomstig van gerepliceerde stedelijke en rurale  
62 populaties en opgegroeid in een gestandaardiseerde laboratorium-omgeving, bloot aan elkaar  
63 volgens een experimenteel design waarbij alle combinaties van herkomst van prooi en  
64 predator werden getest. We kwantificeerden telkens de predatiesnelheid en functionele  
65 respons-kenmerken.
- 66 4. Stedelijke waterjufferlarven vertoonden een hogere ontmoetings- en predatiesnelheid dan  
67 larven afkomstig uit rurale gebieden wanneer ze blootgesteld werden aan rurale *Daphnia*,  
68 maar niet wanneer ze blootgesteld werden aan *Daphnia* afkomstig uit de stad. Deze bevinding  
69 wijst op een cryptische evo-naar-eco terugkoppeling, waarbij evolutie in één van de soorten  
70 het effect van evolutie van de andere soort op de sterkte van hun interactie dempt. De effecten  
71 van evolutie in elke soort apart waren sterk: de scenario's waarin enkel de predator of de  
72 prooi aangepast was aan de stadsomgeving resulteerde in een ca. 250% stijging van  
73 ontmoetingssnelheid en een ca. 25% verhoging van de predatiesnelheid, in vergelijking met  
74 een situatie waarbij een rurale predator met een rurale prooi was gecombineerd.
- 75 5. Deze resultaten leveren uniek bewijs voor een eco-evolutionaire terugkoppeling in steden, en  
76 onderlijnen het belang van een multi-soorten benadering in het onderzoek naar eco-  
77 evolutionaire dynamieken.

78 **INTRODUCTION**

79 Evolution can occur at small temporal and spatial scales, and can lead to trait changes that can  
80 profoundly influence ecology, including population dynamics, interaction strengths, community  
81 composition, and ecosystem functioning (Pantel et al. 2015, Kinnison et al. 2015, Hendry 2016, De  
82 Meester et al. 2019). Such ‘broad sense’ eco-evolutionary feedbacks (Hendry 2017, De Meester et al.  
83 2019) may fundamentally change species interaction dynamics, including predation (De Meester et al.  
84 2019). Most eco-evolutionary dynamics research focuses on consequences of evolution in one species  
85 and in simplified settings, providing proof-of-principle rather than quantifying eco-evolutionary  
86 feedbacks across realistic landscapes (De Meester et al. 2019). Empirical (De Meester et al. 2019) and  
87 modelling (Vanoverbeke et al. 2016, Cortez 2018, Thompson and Fronhofer 2019) studies on eco-  
88 evolutionary feedbacks in which the evolution of multiple interacting species to the same stressor,  
89 hence not in the context of co-evolution, is taken into consideration are very rare,

90         When quantifying the ecological effect of evolution in a species of a community, the effect of  
91 non-equilibrium settings in which only one of the species was allowed to evolve is measured. This  
92 may reflect reality in cases where rates of evolutionary trait change differ among species (Hendry and  
93 Kinnison 1999) or in which interacting populations have a different evolutionary history (e.g. upon  
94 encounter after dispersing into a novel habitat). Such studies provide an estimate of the potential  
95 impact of evolution in a particular species on the interaction strength with other species. However, if  
96 different partners involved in species interactions evolve simultaneously to the same stressor, they  
97 might increase or decrease the net cumulative effect of evolution on their interaction strength. Eco-  
98 evolutionary dynamics research across realistic systems and landscapes urgently requires to test how  
99 eco-evolutionary feedbacks are impacted by the joint evolution of interacting species. Such dynamics,  
100 well-known in the context of co-evolution (where species directly evolve in response to selection  
101 imposed by each other), are less understood in the context of independent evolutionary responses in  
102 multiple species to environmental change, including human-induced selection pressures (Urban et al.  
103 2016).

104 A key challenge in identifying and quantifying eco-evolutionary dynamics in nature is that the  
105 effects of evolution are often compensatory, counteracting environmentally-induced phenotypically  
106 plastic changes (Ellner et al. 2011, Kinnison et al. 2015). Evolution in response to an environmental  
107 change thus can result in trait differentiation across space that dampens the ecological changes that are  
108 expect to result from the environmental challenge, and consequently reduces the net ecological impact  
109 across the gradient(Ellner et al. 2011, Kinnison et al. 2015, Urban et al. 2020). This makes the  
110 detection of eco-evolutionary feedbacks less straightforward, as it may be the absence of any  
111 ecological change across environmental gradients that indicates eco-evolutionary feedbacks (Urban et  
112 al. 2020). In a multispecies context, this dampening effect of evolution can also extend to evolution in  
113 one species buffering the ecological effects of evolution in another species (Nadeau and Urban 2019).  
114 The combined effect of evolutionary trait change in multiple species will thus not necessarily result in  
115 an increasing net contribution of trait evolution to ecology. Evolution in one species may dampen the  
116 evo-to-eco effects that originate from the evolution in the other species. Indeed, the net effect of trait  
117 evolution in multiple species on ecology might be smaller than the effect of evolution in a single  
118 species, and may even be reduced to zero, thereby masking the ecological effect of the evolution in  
119 the single species. This would generate eco-evolutionary dynamics that are ‘cryptic’ (Kinnison et al.  
120 2015).

121 Urbanization is associated with strong and novel abiotic and biotic selection pressures  
122 (Grimm et al. 2008, Pickett et al. 2016) and can lead to rapid non-adaptive (i.e. as a consequence of  
123 neutral genetic drift, gene flow, and chance mutations) and adaptive (i.e. in response to natural and  
124 sexual selection) evolution, as shown by the recent burst of studies covering plants, animals, and  
125 micro-organisms (Johnson and Munshi-South 2017, Szulkin et al. 2020). Several studies have  
126 reported adaptive genetic responses to urban warming (Diamond et al. 2017), pollution (Whitehead et  
127 al. 2017), and fragmentation (Cheptou et al. 2017). Given the steep environmental gradients and the  
128 observed patterns of microgeographic adaptation, cities are excellent model systems to quantify how  
129 evolutionary trait change affects ecological processes and to what extent these eco-evolutionary  
130 feedbacks (‘broad sense’, Hendry 2017, De Meester et al. 2019) meaningfully impact ecosystem

131 functions and services in cities (Alberti et al. 2017, Brans et al. 2020, Des Roches et al. 2020). So far,  
132 however, attempts to quantify these feedbacks in cities are limited (Brans et al. 2017a), and no study  
133 has explored whether urban evolution in multiple species leads to intensified, or potentially dampened  
134 evo-to-eco effects, and thus cryptic eco-evolutionary dynamics.

135 Predation is a key trophic interaction structuring pond communities, for which eco-  
136 evolutionary dynamics have been repeatedly shown (Hairston et al. 2005, Yoshida et al. 2007,  
137 Palkovacs and Post 2008, Bassar et al. 2010). It is well documented that genetic adaptation in prey to  
138 a specific predator can change predator-prey dynamics (Yoshida et al. 2007). It is, however, likely  
139 that adaptation of prey or predator to environmental change, like changes in temperatures, can  
140 similarly induce changes in interaction strengths of the predator-prey interaction (Tüzün and Stoks  
141 2018). Moreover, adaptation of both predator and prey to the same environmental gradient can lead to  
142 additional interactions that may amplify or reduce the net effect of evolution on interaction strengths.  
143 In one example, De Block et al. (2013) indeed reported that the outcome of predator-prey interactions  
144 between nymphal damselfly predators (*Ischnura elegans*) and water flea prey (*Daphnia magna*) did  
145 not only depend on temperature during the predator trials, but also on the latitude of origin of both  
146 predator and prey (animals originated from three source latitudes spanning >1500 km).

147 We here tested the hypothesis that urban evolution of both predator and prey jointly drives the  
148 outcome of their short-term trophic interaction and quantified to what extent this interaction is  
149 compensatory, leading to a cryptic eco-evolutionary feedback. We thereby used a text-book example  
150 of a predator-prey interaction between nymphs of the damselfly *Ischnura elegans* and its prey the  
151 water flea *Daphnia magna* (Begon et al. 2005). We capitalized on the adaptation to urban heat islands  
152 in both damselflies and water fleas in the study area (Flanders, Belgium). The majority of these  
153 responses are linked to the thermal regimes typical for urban areas (Wouters et al. 2017, Brans et al.  
154 2018b). Urban ponds in Flanders are warmer compared to rural ponds (up to 3°C and 4°C warmer in  
155 mean and maximum daily summer temperatures, respectively) and have longer estimated growing  
156 seasons (Brans et al. 2018b). Moreover, urban areas in Flanders experience more intense and longer  
157 heat periods (Lauwaet et al. 2018), which is predicted to be further exacerbated with future climate

158 change (Wouters et al. 2017). Urban evolution of a slower growth rate (in response to relaxed time  
159 constraints for nymphal development during the longer growing seasons in urban areas), and a higher  
160 ability to cope with heat stress in terms of energy availability (in response to the stronger and more  
161 frequent heat waves in urban areas) have been shown in *Coenagrion* damselfly nymphs (Tüzün et al.  
162 2017, Tüzün and Stoks 2021), a genus closely related to *Ischnura*. Urban evolution of a higher heat  
163 tolerance, haemoglobin content, faster pace-of-life, higher level of energy storage molecules and an  
164 altered stress physiology has been documented in the water flea *D. magna* (Brans et al. 2017b, 2018a,  
165 Brans and De Meester 2018).

166         Using predators and prey sampled from replicated urban and rural ponds in the study area, we  
167 first assessed multiple fitness-related traits in both *Ischnura* (development time, heat tolerance, body  
168 mass) and *Daphnia* (body size) in common-garden settings to confirmed previously reported genetic  
169 differentiation between urban and rural populations of both damselflies and water fleas. We then  
170 quantified predation rates of urban and rural damselfly predators, reared under different rural and  
171 urban thermal regimes and being exposed to a five-day heatwave, when cross-exposed to urban and  
172 rural water flea prey, that had undergone the same thermal rearing and heatwave conditions. To get  
173 mechanistic insights in the predation rates, we thereby also directly estimated encounter rate (the  
174 inverse of the time between successive encounters) and handling time, two important determinants of  
175 the functional response of a predator (Jeschke et al. 2002, Begon et al. 2005). The effects of extreme  
176 temperature events, which can be lethal and are fundamentally different from increases in average  
177 temperatures as organisms are challenged at the extremities of their thermal tolerance range (Sinclair  
178 et al. 2012, Sentis et al. 2013a, 2017), may thus trigger evo-to-eco feedbacks shaped by thermal  
179 adaptation. Predation rates often increase with increasing average temperatures, yet decrease rapidly  
180 at extreme temperatures as a result of decreased searching activities and encounter rates, in  
181 combination with longer handling times (Sentis et al. 2013b, Stoks et al. 2017). We predicted that  
182 thermal adaptation to higher temperatures in urban damselfly populations might allow them to sustain  
183 higher predation activities during a heatwave compared to rural predators. Likewise, evolution of a  
184 higher heat tolerance in urban water flea prey might reduce their sensitivity to thermal stress, lowering



185 their vulnerability to predation. We additionally hypothesized that scenarios in which both the  
186 predator and prey evolve to urban habitats lead to predatory-prey interactions that differ from  
187 scenarios in which either only the prey or the predator evolve. More specifically, we predicted that the  
188 net effect of evolution on predator-prey interaction strength might be reduced if both predator and  
189 prey evolve, leading to a cryptic evo-to-eco feedback (i.e. urban evolution to predator-prey  
190 interactions).

## 191 **METHODS**

### 192 **Collecting and rearing of *Ischnura elegans* predator and *Daphnia magna* prey**

193 Both damselfly (*Ischnura elegans*) predators and water flea (*Daphnia magna*) prey originated from  
194 replicated urban and rural ponds in Flanders (Belgium; details in Table S1 and Fig. S1, Appendix -  
195 section A. Methods). To avoid interference from local adaptation to a given pond, as well as the  
196 potential of co-evolution of the predator and prey study populations, we used different sets of urban  
197 and rural ponds for predator and prey. Flanders is densely populated (ca 700 inhabitants/ km<sup>2</sup>, IBZ,  
198 2021). We used percentage built-up area (BA) in the regional surroundings of the pond [based on the  
199 Large-scale Reference Database, LRD (2013)] to assess urbanization levels (urban/rural) of study  
200 populations (as in Brans & De Meester, 2018; Brans et al. 2017a,b, 2018, detailed in Appendix -  
201 section A. Methods). %BU, a metric including housing and building infrastructure but excluding  
202 roads and parking lots, has shown to be a reliable proxy of urbanization as it, in the specific study  
203 region, positively correlates with impervious substrate cover (e.g. roads) and artificial constructions  
204 (e.g. bridges), and is negatively associated with the area of seminatural habitats (Piano et al., 2020). In  
205 addition, rural locations had to meet the criterion of a minimum 20% biologically valuable area  
206 (detailed in Appendix - section A. Methods) to prevent sampling populations in non-urban  
207 agricultural areas.

208 Rearing conditions for both *Ischnura* and *Daphnia* during the pre-experimental generations  
209 are detailed in Appendix (section A. Methods). In short, nymphs hatched from eggs obtained of field-  
210 mated *Ischnura* females from three urban and three rural populations, were individually reared at

211 standardized laboratory conditions (ad libitum daily feeding with *Artemia* nauplii, 14:10 L:D  
212 photoperiod). Nymphs were reared at either 20 or 24°C ( $\pm 0.5^\circ\text{C}$ ) using temperature-controlled water  
213 baths, until reaching the final nymph stage, and then assigned to a specific heatwave treatment (see  
214 further). For *Daphnia*, three clones from each of four urban and four rural populations (total n=24  
215 clones) were kept in the laboratory for several generations; clones were first cultured for two  
216 generations (20°C; 16:8 L:D photoperiod; 100000 cells/mL of the green algae *Acutodesmus obliquus*)  
217 to obviate interference from (grand)maternal effects, after which for each clone, triplicate cohorts (12  
218 individuals in 500 mL dechlorinated tap water) were reared for two experimental generations at 20  
219 and 24°C ( $\pm 0.9^\circ\text{C}$ ) in a temperature-controlled room under standardized conditions (100000 cells/m  
220 *Acutodesmus obliquus*, 14:10 L:D photoperiod, two-daily water refreshment of 80%). New  
221 generations were started up using 2<sup>nd</sup> to 4<sup>th</sup> clutch offspring, which enabled to standardize the number  
222 of generations and time in the lab across all populations. From these cohorts, newborn juveniles were  
223 transferred to the heatwave treatments

224 *Ischnura* nymphs were reared in the common-garden setting for one generation, from egg to  
225 final instar. While we cannot exclude maternal effects to partly shape observed differences among  
226 urban and rural predator nymphs, Shama et al. (2011) showed maternal effects to play a minor role in  
227 shaping life-histories of the same species. Differences in life history traits observed in our experiment  
228 can thus be confidently interpreted as largely reflecting genetic differences. *Daphnia* prey genotypes  
229 were reared for multiple generations under common-garden conditions, ensuring that (grand)maternal  
230 effects do not interfere with genetic differences in determining trait values. *D. magna* reproduces by  
231 cyclical parthenogenesis, so that genotypes can be kept as clonal lineages in the laboratory during  
232 many generations.

## 233 **Experimental design and procedures**

### 234 *General set-up*

235 Both predator and prey were separately reared at 20 or 24°C (mimicking mean rural and urban  
236 summer temperatures for ponds in the region, Brans et al. 2018b, Wouters et al. 2017) and exposed to  
237 a 4°C or 8°C temperature increase to reach heatwave temperatures of 28°C (rural areas) and 32°C

238 (urban areas) resulting in three heatwave treatments (Fig. 1): HW20-28°C (20°C rearing temperature  
239 and 28°C heatwave temperature), HW24-28°C (24°C rearing temperature and 28°C heatwave  
240 temperature), and HW24-32°C (24°C rearing temperature and 32°C heatwave temperature). As we  
241 focused on testing the effects of urbanization background and thermal acclimation on predation rates  
242 under thermally challenging conditions, all animals were exposed to a simulated heatwave. Moreover,  
243 as testing our aims did not require a full factorial design, we did not install two thermal treatments:  
244 individuals reared at 20°C did not undergo a 4°C temperature increase to 24°C, as this does not result  
245 in a thermally challenging condition. A temperature increase from 20°C to 32°C was also not  
246 implemented, as this temperature difference is less commonly observed in the study region. Predation  
247 trials were video-recorded (Sony HDR-CX 240, recorded at 25 fps) to later extract two functional  
248 response parameters (encounter rate and handling time).

249 The full experimental set-up [2 urbanization levels (rural/urban origin) × 2 species  
250 (predator/prey) × 3 heatwave treatments (HW20-28°C/HW24-28°C/HW24-32°C)] resulted in a total  
251 of 12 combinations, which were replicated 12 times (total of 144 predation trials) (Fig. 1).  
252 Independence of replicates was maximized via a randomization across origin of clonal lineages and  
253 *Ischnura* mother identity (i.e. ‘family’) (see Appendix – section A. Methods).

#### 254 *Heatwave treatment and predation trials*

255 Both damselfly nymphs and water flea cohorts (30 newborn individuals, fed *ad libitum* throughout the  
256 heatwave with the green algae *Acutodesmus obliquus*, eliminating possible intraspecific competition)  
257 passed separately through a simulated five-day heatwave of either 28 or 32°C (HW20-28°C/HW24-  
258 28°C/HW24-32°C). At the end of the heatwave period, when any effects of the heatwave on predator  
259 and prey are assumed to be most pronounced, 1h-predation trials took place at the associated heatwave  
260 temperature. This set-up enabled us to measure functional response parameters (encounter rate,  
261 handling time, and predation rate) in a standardized and controlled way without interference of  
262 numerical effects.

263 The procedures related to the heatwave exposures are detailed in Appendix (section A.  
264 Methods). In short, the simulated 5-day heatwaves consisted of a gradual increase in temperature over  
265 the first two days, followed by two days at the peak temperature (detailed in Appendix- section A.  
266 Methods). The medium for the *Daphnia* was renewed on the first and third day of the heatwave period  
267 (with medium incubated at the appropriate water temperature of the specific heatwave condition). At  
268 the end of the heatwave (day five), 20 randomly chosen *Daphnia* of the cohort were inoculated into a  
269 2l aquarium (180×133×126 mm) with fresh medium (at the associated heatwave temperature), and  
270 allowed one hour of acclimatization. Another three randomly chosen *Daphnia* per cohort were  
271 measured for body size (Brans et al. 2017a, see Appendix - section A. Methods). Thereafter, one  
272 damselfly nymph (starved for 24h before the predation trial) of the same heatwave treatment, and  
273 according to experimental combinations represented in Figure 1, was added to the trial aquarium, and  
274 a 60-minute predation trial started. In some cases (n=6 out of 144) *Daphnia* mortality after the  
275 heatwave in a clonal cohort was present, hence <20 (range: 16-19) *Daphnia* could enter the predation  
276 trial After each predation trial, damselflies were scored for CT<sub>MAX</sub> (critical thermal maximum, i.e. the  
277 temperature at which the animal fails to maintain motoric functioning and faints), using the same  
278 procedure as in Op de Beeck et al. (2017), weighted, and sexed (see Appendix - section A. Methods  
279 for details).

## 280 **Response variables**

281 Measurement of the variables to characterize adaptation to the urban environment (heat tolerance,  
282 body mass, and development time for predator, and body size for prey) were conducted based on  
283 standardized measurement protocols (for specifics see Appendix - section A. Methods).

284 Predation rates were expressed as  $N_{\text{eaten}}/N_{\text{initial}}$  of *Daphnia* after one hour in the trial. Using the  
285 video recordings of each trial, we directly calculated two functional response parameters, encounter  
286 rate and handling time based on the three first successful attacks (Jeschke et al. 2002, Novak and  
287 Wootton 2008, Okuyama 2010, Twardochleb et al. 2020). The encounter rate ( $s^{-1}$ ) was calculated as  
288 the inverse of the searching time, which is the length of the time interval from the moment the  
289 predator finished consuming a prey item to the moment a next successful attack took place Note that

290 for predators with negligible attack times, e.g. for sit-and-wait predators such as damselfly nymphs,  
291 calculating encounter rates using only successful attacks is expected to result in reliable encounter  
292 rate estimates (Jeschke et al. 2002). In our experiment, encounter rate was quantified twice per trial:  
293 searching time for the second and third prey item. Note that no searching time was calculated for the  
294 first captured prey, given the time needed to find the first prey is confounded with the time needed for  
295 the predator to familiarize with the new environment. The handling time, defined as the duration that  
296 a predator spends on consuming a captured prey was calculated as the length of the time interval from  
297 the moment of capturing the prey to the moment of finishing prey consumption (three estimates per  
298 trial, handling time of the first three prey items).

### 299 **Statistical analyses**

300 All statistical analyses were conducted with R v3.5.3 (3.5.1. 2018), using the packages ‘lme4’ for  
301 linear models (Bates et al. 2015), ‘lmerTest’ for F-test of fixed effects (error calculation Type III sums  
302 of squares) (Kuznetsova et al. 2017) and ‘emmeans’ for Tukey’s post-hoc pairwise comparisons  
303 following significant interaction effects (Lenth 2020). To meet model assumptions of normality,  
304 encounter rate and handling time were log transformed. All models conformed with assumptions of  
305 homogeneity of variances and regression slopes.

306 Statistical analyses on *Ischnura* body mass, development time and  $CT_{MAX}$ , and *Daphnia* body  
307 size are detailed in Appendix (section A. Methods).

#### 308 *Encounter rate and handling time*

309 We computed a linear mixed-effect model to test for an effect of urbanization background of both  
310 predator and prey, the heatwave treatment, and their interactions, on the two measured functional  
311 response parameters of the predator (see simplified model formula). Additionally, predator sex and  
312 time point of attack were included as fixed effects, and predator body mass and prey size as  
313 covariates. As random effects we included the population of origin of predator and prey (nested in  
314 urbanization background), the family (for *Ischnura*) and clone (for *Daphnia*) nested in population, and  
315 the date of the start of the heatwave (see below for the model syntax in R language). Given encounter

316 rates and handling times were assessed based on the first three successful attacks, a repeated measures  
317 structure for each estimate (two for encounter rate, three for handling time) was included by including  
318 trial ID as an additional random effect.. While both variables can be impacted by prey size and  
319 predator body mass, (Thompson 1978), adding or removing *Daphnia* size and *Ischnura* body mass to  
320 the models did not qualitatively change the results (see Results and Appendix - section B. Results).  
321 We nevertheless kept prey size and predator mass (as covariates) in the final models. This resulted in  
322 following model: response  $\sim$  *Ischnura* urbanization background  $\times$  *Daphnia* urbanization background  
323  $\times$  heatwave treatment + *Ischnura* sex + *Ischnura* mass + *Daphnia* size + time point + (1 | *Ischnura*  
324 population / *Ischnura* mother) + (1 | *Daphnia* population / *Daphnia* clone) + (1 | trial date) + (1 | trial  
325 ID).

#### 326 *Predation rates*

327 We computed a similar linear mixed-effect model as for the functional response parameters to test for  
328 an effect of urbanization of predator and prey, and heatwave treatments on predation rates (fixed  
329 effects: urbanization background of both predator and prey, and the heatwave treatment) (see  
330 simplified model formula). We included the specific wet mass of *Daphnia* and damselfly (corrected  
331 for the number and specific mass of eaten *Daphnia* as before) as covariates for each trial, and  
332 *Ischnura* sex as fixed effect in the analyses. As random effects we included the population of origin of  
333 predator and prey (nested in urbanization background), the family (for *Ischnura*) and clone (for  
334 *Daphnia*), nested in population, and the date of the start of the heatwave. For the same reason as  
335 mentioned for handling time and encounter rate, we included predator mass and prey size as  
336 covariates. The model without mass and size did not qualitatively change the results; see Results and  
337 Appendix - section B. Results (but see below for an additional model upon the detection of a  
338 significant effect of prey size on predation rates). Testing models using *Daphnia* body mass instead of  
339 size did not qualitatively change the results (see Results and Appendix - section B. Results). This  
340 resulted in following model: predation rate  $\sim$  *Ischnura* urbanization background  $\times$  *Daphnia*  
341 urbanization background  $\times$  heatwave treatment + *Ischnura* sex + *Ischnura* mass + *Daphnia* size + (1 |  
342 *Ischnura* population / *Ischnura* mother) + (1 | *Daphnia* population / *Daphnia* clone) + (1 | trial date).

343 Finally, to account for potential effects of geography beyond rural and urban designations on  
344 the tested response variables (encounter rate, handling time, predation rate), we constructed separate  
345 models with the same structures as described above, that additionally included latitude and longitude  
346 of both predator and prey populations as covariates. As these models resulted in qualitatively similar  
347 effects of urbanization levels of the prey and predator (and their interaction), we exclude these  
348 covariates from the final models, but report the full statistical results in the Appendix (section B.  
349 Results)

## 350 **RESULTS**

351 Results on *Daphnia* body size and *Ischnura* development time, heat tolerance ( $CT_{MAX}$ , Critical  
352 Thermal Maximum), and body mass are detailed in Appendix (section B1. Results, Figures S2- S5,  
353 Tables S2 and S3), and confirm previously reported differences between urban and rural populations  
354 of predator and prey species. In short, urban *Ischnura* predator nymphs had a ca. 15 day longer  
355 development time ( $p=0.001$ ) (see Tüzün et al. 2017) and ca. 5% higher body mass ( $p=0.008$ )  
356 compared to rural ones, whereas they did not differ in  $CT_{MAX}$  ( $p=0.834$ ). Urban *Daphnia* prey were ca.  
357 5% smaller ( $p=0.036$ ) compared to rural *Daphnia* (see Brans et al. 2017b, Brans and De Meester  
358 2018).

### 359 **Encounter rate and handling time**

360 Encounter rates ( $s^{-1}$ , the inverse of time used for searching prey), were significantly impacted by the  
361 interaction between urbanization backgrounds of predator and prey ( $F_{1/1231.6}=16.111$ ,  $p<0.001$ , Table  
362 1A, Fig. 2A,B). Urban *Ischnura* had a higher encounter rate compared to rural *Ischnura* in the  
363 presence of rural *Daphnia* (post-hoc test,  $p=0.007$ , Fig. 2B, Table S4, Appendix - section B2.  
364 Results), but not in the presence of urban *Daphnia* ( $p=0.104$ , Fig. 2B, Table S4). Rural *Daphnia* were  
365 encountered faster than urban *Daphnia* by urban *Ischnura* ( $p=0.023$ , Fig. 2B, Table S4), while the  
366 opposite pattern was observed in the presence of rural *Ischnura* ( $p=0.025$ , Fig. 2B, Table S4). Results  
367 (Fig. 2A) suggests that this interaction effect is most strongly developed in the heatwave treatment  
368 mimicking urban conditions (HW24-32°C, Table 1A, see also Table S4).

369 Handling times (s, the duration a predator spends on prey consumption) were jointly driven  
370 by both the urbanization backgrounds of the prey and predator, and the heatwave treatment (cf.  
371 Heatwave  $\times$  Urbanization predator  $\times$  Urbanization prey,  $F_{2/146.3}=5.090$ ,  $p=0.007$ , Table 1B, Fig. 2D,E).  
372 In the treatment mimicking urban conditions with a strong heatwave (HW24-32°C), urban *Ischnura*  
373 had a shorter handling time compared to rural *Ischnura* in the presence of rural *Daphnia* (post-hoc  
374 test,  $p=0.033$ , Table S5, Appendix - section B2. Results), but had a similar handling time in the  
375 presence of urban *Daphnia* ( $p=0.502$ , Table S5). In this heatwave, rural *Daphnia* were handled faster  
376 than urban *Daphnia* in the presence of urban *Ischnura* ( $p=0.006$ , Table S5), whereas handling times  
377 were similar for rural and urban *Daphnia* eaten by rural *Ischnura* ( $p=0.855$ , Table S5).

378 Encounter rates decreased ( $F_{1/144.49}=5.552$ ,  $p=0.020$ , Table 1A) and handling times  
379 ( $F_{2/294.6}=7.002$ ,  $p=0.001$ , Table 1B) increased with successive attacks.

### 380 **Predation rate**

381 Predation rates ( $N_{\text{eaten}}/N_{\text{initial}} \cdot h^{-1}$ , ratio of eaten prey to initial prey) were significantly impacted by the  
382 interaction between the urbanization backgrounds of predator and prey ( $F_{1/121.895}=5.912$ ,  $p=0.016$ ,  
383 Table 1C, Fig. 2G,H). Urban *Ischnura* had a higher predation rate compared to rural *Ischnura* in the  
384 presence of rural *Daphnia* (post-hoc test,  $p=0.021$ , Fig. 2H, Table S6, Appendix - section B2.  
385 Results), but both had a similar predation rate in the presence of urban *Daphnia* ( $p=0.680$ , Fig. 2H,  
386 Table S6). In reverse, rural *Daphnia* suffered higher predation than urban *Daphnia* in the presence of  
387 urban *Ischnura* ( $p=0.034$ , Fig. 2H, Table S6), but not in the presence of rural *Ischnura* ( $p=0.718$ , Fig.  
388 2H, Table S6). The heatwave treatment affected the predation rates ( $F_{2/107.604}=6.687$ ,  $p=0.002$ , Table  
389 1, Fig. 2G): predation rates were higher at HW20-28°C than at HW24-28°C (post-hoc test,  $p=0.019$ ,  
390 Table S6) and HW24-32°C ( $p=0.008$ , Table S6), but did not differ between the latter two heatwaves  
391 ( $p=0.984$ , Table S6). Predation rates increased with smaller prey size ( $\beta=-0.048 \pm 0.014$ ,  $p=0.003$ ,  
392 Table 1C).

### 393 **DISCUSSION**



394 By applying a two-species common-garden approach, we demonstrated how independent evolution in  
395 response to urbanization in both damselfly predators and water flea prey affects their trophic  
396 interaction under thermal stress. Notably, our results provide a unique example of a cryptic evo-to-eco  
397 feedback ('broad sense' *sensu* De Meester et al. 2019, Hendry 2017) in the context of urbanization, in  
398 which urban evolution of predator and prey only leads to differences in encounter and predation rates  
399 when the urbanization backgrounds of predator and prey do not match. In the next paragraphs we  
400 detail our findings and elaborate on potential mechanisms underlying them.

401 *A cryptic evo-to-eco feedback: urban evolution in the prey dampens the ecological effect of urban*  
402 *evolution in the predator in a thermally challenging environment*

403 While eco-evolutionary feedbacks in which evolution of one species to a variable extent dampens the  
404 effects of evolution in another species are well-documented in cases of co-evolution (geographic  
405 mosaic of co-evolution, Thompson 2005; host-parasite co-evolution, Decaestecker et al. 2007;  
406 sympatric predator-prey interactions, Hairston et al. 2005), our results provide a rare demonstration of  
407 these feedbacks in response to independent evolution of two species as they adapt to a recurrent,  
408 common geographic gradient. This may lead to 'cryptic eco-evolutionary dynamics' (Kinnison et al.  
409 2015), whereby the ecological effects of evolution are only revealed in common-garden experiments  
410 where predators and prey from different backgrounds are confronted with each other. More  
411 specifically, encounter rates of urban damselflies were higher compared to rural damselflies when  
412 encountering rural *Daphnia*, but similar when feeding on urban *Daphnia*. Conversely, encounter rates  
413 with urban *Daphnia* were lower compared to those with rural *Daphnia* in confrontation with an urban  
414 predator, while the opposite was observed when confronted with a rural predator. Patterns in handling  
415 times were similar, but less pronounced, and strongest in the thermal conditions mimicking urban  
416 conditions most (HW24-32°C, Fig. 2D). Both functional response parameters, here estimated based  
417 on the first three prey captured, culminated in a similar pattern for the overall predation rates: urban  
418 damselflies had higher predation rates compared to rural predators, but only when encountering rural  
419 water fleas, and not urban water fleas. In reverse, rural *Daphnia* were preyed upon more compared to  
420 urban *Daphnia* in the presence of urban predators, but not rural predators. Urban evolution in the prey

421 thus dampened the ecological effects of urban evolution in the predators. The combined effect of  
422 evolution in both predator and prey concealed the effect of evolution in each of the interactors,  
423 thereby generating an ecological feedback ('broad sense', Hendry 2017, De Meester et al. 2019) from  
424 evolution which is consistent with a scenario of cryptic eco-evolutionary dynamics.

425 Our study highlights local adaptation and an eco-evolutionary feedback in the context of  
426 urbanization. Our results are in line with observations along latitudinal thermal gradients, where  
427 striking interaction effects of the genetic background of damselfly predators and water flea prey,  
428 originating from different latitudes, were reported (De Block et al. 2013). Predation rates of low-  
429 latitude damselflies on low-latitude *Daphnia* were much higher than those of high-latitude damselflies  
430 on high-latitude *Daphnia*. We show that genotype  $\times$  genotype interactions in this classical predator-  
431 prey model system also occur across local thermal gradients, where dispersal-mediated mixing of  
432 predators and prey from different evolutionary backgrounds (i.e. urban–rural) are likely more frequent  
433 compared to mixing across latitudes. Notably, the here reported cryptic eco-evolutionary feedback is  
434 not negligible. Scenarios where one of the interacting species did not evolve in response to  
435 urbanization (e.g. as a consequence of reduced evolutionary potential or because of recent  
436 immigration from a different background) would lead up to a ca 250% and 25% increase in encounter  
437 and predation rates, respectively, compared to rural-rural and urban-urban conditions (Fig. 2C,I).

438 Compared to most other studies on eco-evolutionary feedbacks, we here worked with natural  
439 populations that evolved in situ under the natural selection regimes thereby working with realistic  
440 selection landscapes. Yet, further increasing realism during the predation trials would be an important  
441 next step. By having done the trials at one, ad libitum density, at a given light level and in the absence  
442 of submerged macrophytes, the absolute predation rates we observed may have been different  
443 compared to those in situ. Yet, it is important to note that these environmental factors (transparency,  
444 macrophyte cover, *Daphnia* density) do not systematically differ between urban and rural ponds in  
445 our study area (Engelen 2017). Thermal evolution likely is a driving factor for the observed  
446 evolutionary morphological, life history, and physiological trait differences (Brans et al. 2017b,  
447 2018b, Tüzün et al. 2017, Tüzün and Stoks 2021), as well as for the here reported patterns in

448 predation rates – an ecologically relevant trait. It is therefore likely that running the predation trials  
449 under different environmental conditions in terms of light level, macrophyte presence or *Daphnia*  
450 densities would not change the patterns of relative differences in predation rates in function of the  
451 urbanization backgrounds of both predator and prey. Nevertheless, given the complexity of urban  
452 systems, we cannot fully exclude that other potential, non-temperature related, factors may contribute  
453 to the here observed responses.

454         Using a single prey density, we estimated the two functional response parameters directly  
455 from observations, instead of using the more extended method of measuring predation rates across  
456 prey densities and fitting a functional response model to estimate parameter values. Previous studies  
457 have reported that both methods can yield similar results, but can also differ depending on predator  
458 satiation level or density-mediated changes in predator behaviour (Novak and Wootton 2008, Sentis et  
459 al. 2013b). It would thus be interesting to conduct a similar study using the extended method to better  
460 understand the role of prey density in eco-evo feedbacks.

#### 461 *Underlying mechanisms – evolutionary responses to urbanization*

462 The cryptic eco-evolutionary feedback reported here is linked to urban evolution of both interaction  
463 partners. Given that the damselfly families and *Daphnia* clones used in this study were isolated from  
464 different localities, it is highly unlikely that our findings are the result of direct co-evolution. Rather it  
465 is related to independent trait evolution to urban environments in interacting species. Urban evolution  
466 in both damselflies and in *Daphnia* is driven by, amongst others, the urban heat island effect (Brans et  
467 al. 2017b, 2018b, Tüzün et al. 2017, Tüzün and Stoks 2021). In the current study we did not observe  
468 that urban *Ischnura* damselflies evolved a higher heat tolerance compared to rural damselflies, yet  
469 they had a slower development and a higher body mass compared to rural damselflies (see Appendix  
470 – section B. Results), in line with earlier on a closely related species (*Coenagrion puella*, Tüzün et al.  
471 2017). In contrast, the urban *Daphnia* used in the present study were smaller than rural ones, again in  
472 line with earlier work (Brans et al. 2017b, Brans and De Meester 2018). Urban evolution thus both  
473 involved a phenological mechanism (predator: countergradient variation as a consequence of longer

474 growing seasons in urban ponds, Tüzün et al. 2017, Brans et al. 2018b), and direct selection for  
475 warming tolerance (temperature-size rule, Daufresne et al. 2009, Brans et al. 2017b).

476 While body size of both predators and prey evolved in response to urbanization, it cannot  
477 explain the observed patterns, as our analyses included body mass (or size) of both species as a  
478 covariate. Rather, our results are potentially related to acquired energy reserves and activity levels.  
479 Earlier work has shown that urban *Daphnia* have higher levels of energy reserves (fat, protein, and  
480 carbohydrates, Brans et al. 2018a) and a faster pace-of-life (e.g. faster development rate, Brans and  
481 De Meester 2018) than rural *Daphnia*. These traits are likely linked to a higher movement and grazing  
482 activity to increase energy intake (Réale et al. 2010). Similarly, Tüzün & Stoks (2021) recently  
483 showed that immune-challenged urban damselfly nymphs (*C. puella*) exposed to simulated heatwaves  
484 suffered less from heatwave-induced depletions in energy availability compared to rural damselfly  
485 nymphs. This suggests that urban damselfly populations too have evolved bio-energetic compensatory  
486 mechanisms to better cope with heat stress in the city (Tüzün & Stoks, 2021), and these mechanisms  
487 might similarly be linked to a higher foraging activity. The combined research on this study systems  
488 tentatively suggests that both predators and prey have evolved changes in activity and feeding  
489 behavior in response to urbanization. While damselflies are commonly sit-and-wait predators,  
490 increased activity (e.g. walks, number of attacks, head turns) in urban populations could increase their  
491 predation efficiency and thus food intake in comparison to a less active rural predator (Gerritsen and  
492 Strickler 1977, Werner and Anholt 1993). In contrast, urban *Daphnia* that are more active and have  
493 more energy reserves can spend more time in faster longer distance ‘cruising’ behavior, compared to a  
494 rural prey, that might be confined to typical slower, local, vertical ‘hop-sink’ movements. While  
495 damselflies are usually sit-and-wait predators, even slight changes in activity patterns of both predator  
496 and prey may thus impact encounter rates and successful attack rates (Pawar et al. 2012, Dell et al.  
497 2014), in such a way that they are higher between urban predators and rural prey and vice versa (one  
498 interactor is less spatially confined), compared to reduced encounter, and thus predation, rates when  
499 predators paired with prey from the same evolutionary background (urban-urban: both interactors are  
500 active, but elude each other; rural-rural: both interactors are less active and more spatially confined)

501 (McCauley et al. 1993). Note that while we cautiously proposed a number of plausible mechanisms  
502 related mainly to energy acquisition and activity patterns that could be driving the here observed  
503 patterns, these clearly need further detailed studies for confirmation.

#### 504 *Conclusions & Future directions*

505 By explicitly taking a multispecies perspective on eco-evolutionary dynamics, carrying out predation  
506 trials in a common-garden setting in which we manipulated the genetic background of both predator  
507 and prey, we here document a unique example of a strong, yet cryptic evo-to-eco feedback. Notably,  
508 we revealed this cryptic feedback across a strong selection gradient associated with urbanization.  
509 Encounter and predation rates are similar for urban predators feeding on urban prey and for rural  
510 predators and rural prey, misleadingly suggesting no evolution occurred in urban ponds. Yet strong  
511 effects of evolution are revealed in combinations where only one of the interactors evolved in  
512 response to urbanization. Such cryptic eco-evolutionary interactions are important, as they imply that  
513 strong deviations from expectations might be occurring, here more than a doubling of predation rates,  
514 in case interacting species would not have the same opportunity to evolve (e.g. due to genetic  
515 constraints). Damselflies are efficient predators on *Daphnia*. As a result, mismatches in degree of  
516 urban evolution resulting in higher predation rates might potentially substantially reduce population  
517 sizes of *Daphnia*. Large-bodied *Daphnia*, in turn, are highly efficient grazers on algae compared to  
518 other zooplankton (Chislock et al. 2013, Gianuca et al. 2016). Reduced population sizes of large-  
519 bodied *Daphnia* might thus result in a strongly reduced top-down control of phytoplankton. Given the  
520 urban heat-island effect combined with nutrient enrichment favor the formation of (toxic) algal  
521 blooms (Waajen et al. 2014), such evolutionary mismatches may thus have important consequences.  
522 To inform the extent to which urban eco-evolutionary feedbacks dampen or amplify ecosystem-wide  
523 changes, such as algal blooms (Des Roches et al. 2020), we propose larger outdoor mesocosms or  
524 transplant experiments with fully crossed urban and rural predator-prey combinations, whereby more  
525 natural settings (e.g. predator and prey are interacting throughout the heatwave period) are better  
526 mimicked (Brans et al. 2020).

527           The here studied example and other cases of cryptic evo-to-eco feedbacks also illustrate why  
528 eco-evolutionary dynamics have often been overlooked in the past (Kinnison et al. 2015). Finally, our  
529 results demonstrate the importance of quantifying the effect of evolution of multiple species in  
530 communities when studying eco-evolutionary dynamics (De Meester et al. 2019). The effects of  
531 evolution in multiple species likely often will not simply add up, and might in some cases actually  
532 cancel each other out, as was observed in the present study. It is important to study whether and to  
533 what extent evolution of multiple species leads to additive, synergistic or compensatory effects on  
534 eco-evolutionary dynamics in other systems as it will improve our forecast on how communities of  
535 evolving and dispersing species will respond to the challenges of the Anthropocene (Urban et al.  
536 2016).

537

538

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545

### 546 **Conflict of interest**

547 Authors declare no competing interest.

548

549 **Author contributions statement:** KIB, NT, LDM and RS designed the study; NT and KIB collected  
550 the data; NT, KIB, and AS analyzed the data; KIB wrote the first draft of the manuscript and, together

551 with NT, led the writing of the manuscript and subsequent revisions. All authors contributed critically  
552 to the various drafts of the manuscript.

553

554 **Data availability statement:** Data supporting the results of this manuscript are archived at figshare:

555 <https://doi.org/10.6084/m9.figshare.14252948> (Brans et al. 2021).

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558 **References**

- 559 Alberti, M. et al. 2017. Urban driven phenotypic changes: empirical observations and theoretical  
560 implications for eco-evolutionary feedback. - *Philos. Trans. R. Soc. B Biol. Sci.* 372: 20160029.
- 561 Bassar, R. D. et al. 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. - *Proc.*  
562 *Natl. Acad. Sci. U. S. A.* 107: 3616–21.
- 563 Bates, D. et al. 2015. Fitting linear mixed-effects models using lme4. - *J. Stat. Softw.* 67: 1–48.
- 564 Begon, M. et al. 2005. *Ecology: From Individuals to Ecosystems*. Blackwell.
- 565 Brans, K. I. and De Meester, L. 2018. City life on fast lanes: Urbanization induces an evolutionary  
566 shift towards a faster lifestyle in the water flea *Daphnia*. - *Funct. Ecol.* 32: 2225–2240.
- 567 Brans, K. I. et al. 2017a. Eco-evolutionary dynamics in urbanized landscapes: evolution, species  
568 sorting and the change in zooplankton body size along urbanization gradients. - *Philos. Trans. R.*  
569 *Soc. B Biol. Sci.* 372: 20160030.
- 570 Brans, K. I. et al. 2017b. The heat is on: Genetic adaptation to urbanization mediated by thermal  
571 tolerance and body size. - *Glob. Chang. Biol.* 23: 5218–5227.
- 572 Brans, K. I. et al. 2018a. Urbanization drives genetic differentiation in physiology and structures the  
573 evolution of pace-of-life syndromes in the water flea *Daphnia magna*. - *Proc. R. Soc. B Biol.*  
574 *Sci.* 285: 20180169.
- 575 Brans, K. I. et al. 2018b. Urban hot-tubs: Local urbanization has profound effects on average and  
576 extreme temperatures in ponds. - *Landsc. Urban Plan.* 176: 22–29.
- 577 Brans, K. I. et al. 2020. Evolutionary Dynamics of Metacommunities in Urbanized Landscapes. - In:  
578 Szulkin, M. et al. (eds), *Urban Evolutionary Biology*. Oxford University Press, pp. 175–196.
- 579 Brans, K. I. et al. 2021. Cryptic eco-evolutionary feedback in the city: urban evolution of prey  
580 dampens the effect of urban evolution of the predator. Data from: Figshare online depository,  
581 <https://doi.org/10.6084/m9.figshare.14252948>
- 582 Cheptou, P.-O. et al. 2017. Adaptation to fragmentation: evolutionary dynamics driven by human  
583 influences. - *Philos. Trans. R. Soc. B Biol. Sci.* 372: 20160037.
- 584 Chislock, Michael F. Sarnelle, O. et al. 2013. Do high concentrations of microcystin prevent *Daphnia*  
585 control of phytoplankton? - *Water Res.* 47: 1960–1971.
- 586 Cortez, M. H. 2018. Genetic variation determines which feedbacks drive and alter predator–prey eco-  
587 evolutionary cycles. - *Ecol. Monogr.* 88: 353–371.
- 588 Daufresne, M. et al. 2009. Global warming benefits the small in aquatic ecosystems. - *Proc. Natl.*  
589 *Acad. Sci. U. S. A.* 106: 12788–12793.
- 590 De Block, M. et al. 2013. Local genetic adaptation generates latitude-specific effects of warming on  
591 predator-prey interactions. - *Glob. Chang. Biol.* 19: 689–696.
- 592 De Meester, L. et al. 2019. Analysing eco-evolutionary dynamics—The challenging complexity of the  
593 real world. - *Funct. Ecol.* 33: 43–59.
- 594 Decaestecker, E. et al. 2007. Host–parasite ‘Red Queen’ dynamics archived in pond sediment. -  
595 *Nature* 450: 870–873.
- 596 Dell, A. I. et al. 2014. Temperature dependence of trophic interactions are driven by asymmetry of  
597 species responses and foraging strategy. - *J. Anim. Ecol.* 83: 70–84.
- 598 Des Roches, S. et al. 2021. Socio-eco-evolutionary dynamics in cities. - *Evol. Appl.* 14: 248–267.



- 599 Diamond, S. E. et al. 2017. Rapid evolution of ant thermal tolerance across an urban-rural temperature  
600 cline. - *Biol. J. Linn. Soc.* 121: 248–257.
- 601 Ellner, S. P. et al. 2011. Does rapid evolution matter? Measuring the rate of contemporary evolution  
602 and its impacts on ecological dynamics. - *Ecol. Lett.* 14: 603–614.
- 603 Engelen, J. M. T. 2017. The impact of urbanization on ponds and their zooplankton communities.  
604 PhD Dissertation. KU Leuven.
- 605 Gerritsen, J. and Strickler, J. R. 1977. Encounter Probabilities and Community Structure in  
606 Zooplankton: a Mathematical Model. - *J. Fish. Res. Board Canada* 34: 73–82.
- 607 Gianuca, A. T. et al. 2016. Disentangling the effect of body size and phylogenetic distances on  
608 zooplankton top-down control of algae. - *Proc. R. Soc. B Biol. Sci.* 283: 20160487.
- 609 Grimm, N. B. et al. 2008. The changing landscape: Ecosystem responses to urbanization and pollution  
610 across climatic and societal gradients. - *Front. Ecol. Environ.* 6: 264–272.
- 611 Hairston, N. G. et al. 2005. Rapid evolution and the convergence of ecological and evolutionary time.  
612 - *Ecol. Lett.* 8: 1114–1127.
- 613 Hendry, A. P. 2016. *Eco-evolutionary dynamics*. - Princeton University Press.
- 614 Hendry, A. P. and Kinnison, M. T. 1999. Perspective: The Pace of Modern Life: Measuring Rates of  
615 Contemporary Microevolution. - *Evolution*. 53: 1637.
- 616 Jeschke, J. M. et al. 2002. Predator functional responses: Discriminating between handling and  
617 digesting prey. - *Ecol. Monogr.* 72: 95–112.
- 618 Johnson, M. T. J. and Munshi-South, J. 2017. Evolution of life in urban environments. - *Science*. 358:  
619 eaam8327.
- 620 Kinnison, M. T. et al. 2015. Cryptic eco-evolutionary dynamics. - *Ann. N. Y. Acad. Sci.* 1360: 120–  
621 144.
- 622 Kuznetsova, A. et al. 2017. lmerTest package: Tests in linear mixed effects models. - *J. Stat. Softw.*  
623 82: 1–26.
- 624 Lenth, R. 2020. emmeans : Estimated Marginal Means, aka Least-Squares Means. - R Packag. version  
625 1.15-15.
- 626 LRD. 2013. Large-scale Reference Database, an object-oriented reference map of Flanders. Flanders  
627 Information Agency
- 628 McCauley, E. et al. 1993. Dynamics of age-structured and spatially structured predator-prey  
629 interactions: individual-based models and population-level formulations. - *Am. Nat.* 142: 412–  
630 442.
- 631 Nadeau, C. P. and Urban, M. C. 2019. Eco-evolution on the edge during climate change. - *Ecography*.  
632 42: 1280–1297.
- 633 Novak, N. and Wootton, J. T. 2008. Estimating nonlinear interaction strength: an observation-based  
634 method for species-rich food webs. - *Ecology* 89: 2083–2089.
- 635 Okuyama, T. 2010. Prey density-dependent handling time in a predator-prey model. - *Community*  
636 *Ecol.* 11: 91–96.
- 637 Op de Beeck, L. et al. 2017. Integrating both interaction pathways between warming and pesticide  
638 exposure on upper thermal tolerance in high- and low-latitude populations of an aquatic insect. -  
639 *Environ. Pollut.* 224: 714–721.
- 640 Palkovacs, E. P. and Post, D. M. 2008. Eco-evolutionary interactions between predators and prey: Can

641 predator-induced changes to prey communities feed back to shape predator foraging traits? -  
642 Evol. Ecol. Res. 10: 699–720.

643 Pantel, J. H. et al. 2015. Rapid local adaptation mediates zooplankton community assembly in  
644 experimental mesocosms. - Ecol. Lett. 18: 992–1000.

645 Pawar, S. et al. 2012. Dimensionality of consumer search space drives trophic interaction strengths. -  
646 Nature 486: 485–489.

647 Pickett, S. T. A. et al. 2016. Evolution and future of urban ecological science: ecology *in*, *of*, and *for*  
648 the city. - Ecosyst. Heal. Sustain. 2: e01229.

649 R Core Team. 2018. R: A language and environment for statistical computing. Vienna, Austria.  
650 <https://www.R-project.org>.

651 Réale, D. et al. 2010. Personality and the emergence of the pace-of-life syndrome concept at the  
652 population level. - Philos. Trans. R. Soc. B Biol. Sci. 365: 4051–4063.

653 Sentis, A. et al. 2013a. Effects of simulated heat waves on an experimental plant-herbivore-predator  
654 food chain. - Glob. Chang. Biol. 19: 833–842.

655 Sentis, A. et al. 2013b. Parsing handling time into its components: Implications for responses to a  
656 temperature gradient. - Ecology 94: 1675–1680.

657 Sentis, A. et al. 2017. Non-additive effects of simulated heat waves and predators on prey phenotype  
658 and transgenerational phenotypic plasticity. - Glob. Chang. Biol. 23: 4598–4608.

659 Shama, L. N. S. et al. 2011. Latitudinal and voltinism compensation shape thermal reaction norms for  
660 growth rate. - Mol. Ecol. 20: 2929–2941.

661 Sinclair, B. J. et al. 2012. Variation in thermal performance among insect populations. - Physiol.  
662 Biochem. Zool. 85: 594–606.

663 Sokal, R. R., & Rohlf, J. F. 2001. Biometry - The principles and practice of statistics in biological  
664 research (3rd ed.). New York, NY: W.H. Freeman and Company.

665 Stoks, R. et al. 2017. Daily temperature variation and extreme high temperatures drive performance  
666 and biotic interactions in a warming world. - Curr. Opin. Insect Sci. 23: 35–42.

667 Szulkin, M. et al. 2020. Urban evolutionary biology. Oxford University Press.

668 Thompson, D.J. 1978. Prey size selection by larvae of the damselfly *Ischnura elegans* (Odonata). - J.  
669 Anim. Ecol. 47: 769–785.

670 Thompson, J. N. 2005. The geographic mosaic of coevolution. - University of Chicago Press.

671 Thompson, P. L. and Fronhofer, E. A. 2019. The conflict between adaptation and dispersal for  
672 maintaining biodiversity in changing environments. - Proc. Natl. Acad. Sci. U. S. A. 116:  
673 21061–21067.

674 Tüzün, N. and Stoks, R. 2018. Evolution of geographic variation in thermal performance curves in the  
675 face of climate change and implications for biotic interactions. - Curr. Opin. Insect Sci. 29: 78–  
676 84.

677 Tüzün, N. and Stoks, R. 2021. Lower bioenergetic costs but similar immune responsiveness under a  
678 heatwave in urban compared to rural damselflies. - Evol. Appl. 14: 24–35.

679 Tüzün, N. et al. 2017. Microgeographic differentiation in thermal performance curves between rural  
680 and urban populations of an aquatic insect. - Evol. Appl. 10: 1067–1075.

681 Twardochleb, L. A. et al. 2020. Foraging strategy mediates ectotherm predator–prey responses to  
682 climate warming. - Ecology 101: e03146.

- 683 Urban, M. C. et al. 2016. Improving the forecast for biodiversity under climate change. - Science.  
684 353: aad8466.
- 685 Vanoverbeke, J. et al. 2016. Community assembly is a race between immigration and adaptation: eco-  
686 evolutionary interactions across spatial scales. - Ecography. 39: 858–870.
- 687 Waajen, G. W. A. M. et al. 2014. Eutrophic urban ponds suffer from cyanobacterial blooms: Dutch  
688 examples. - Environ. Sci. Pollut. Res. 21: 9983–9994.
- 689 Werner, E. E. and Anholt, B. R. 1993. Ecological consequences of the trade-off between growth and  
690 mortality rates mediated by foraging activity. - Am. Nat. 142: 242–272.
- 691 Whitehead, A. et al. 2017. When evolution is the solution to pollution: Key principles, and lessons  
692 from rapid repeated adaptation of killifish (*Fundulus heteroclitus*) populations. - Evol. Appl. 10:  
693 762–783.
- 694 Wouters, H. et al. 2017. Heat stress increase under climate change twice as large in cities as in rural  
695 areas: A study for a densely populated midlatitude maritime region. - Geophys. Res. Lett. 44:  
696 8997–9007.
- 697 Yoshida, T. et al. 2007. Cryptic population dynamics: Rapid evolution masks trophic interactions. -  
698 PLoS Biol. 5: 1868–1879.
- 699

700 **Tables**

701 **Table 1.** Linear mixed-effect model results for (A) encounter rate ( $s^{-1}$ , log-transformed), (B) handling  
702 time (s, log-transformed), and (C) predation rate (ratio of  $N_{\text{eaten}}/N_{\text{initial}} \cdot h^{-1}$ ) in response to the heatwave  
703 treatment (HW; HW20-28°C, HW24-28°, HW24-32°C), and urbanization backgrounds (urban/rural)  
704 of both *Ischnura* predators and *Daphnia* prey. The time point of successful attack (first, second, third),  
705 prey body size (mm) was included as fixed effect for encounter rate and handling time. For all  
706 variables, sex of the predator was included as fixed effect and predator body mass (mg, corrected for  
707 number and mass of *Daphnia* eaten at the end of the trial) and prey size (mm) as covariates, as well as  
708 the random nested structure of *Daphnia* clone and *Ischnura* family (mother identity) nested in  
709 population, and population nested in urbanization category for both interactors. For all three variables  
710 heatwave date (i.e. date of the start of the heatwave) was added as random effect to the model.

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<b>A. Encounter rate (<math>s^{-1}</math>)</b>	<b>NumDF</b>	<b>DenDF</b>	<b>F</b>	<b>P</b>
Heatwave treatment (HW)	2	127.2	2.289	0.105
Urbanization predator	1	7.0	0.930	0.367
Urbanization prey	1	9.6	0.014	0.909
HW × Urbanization predator	2	130.3	0.593	0.554
HW × Urbanization prey	2	131.3	0.149	0.862
Urbanization predator × Urbanization prey	1	131.6	16.111	<b>&lt;0.001</b>
HW × Urbanization predator × Urbanization prey	2	134.3	2.675	0.073
Time point attack	1	146.0	5.536	<b>0.020</b>
Sex predator	1	142.2	0.001	0.979
Prey ( <i>Daphnia</i> ) size (mm)	1	117.1	0.092	0.762
Predator ( <i>Ischnura</i> ) body mass (mg, corrected)	1	138.0	1.277	0.260
<b>B. Handling time (s)</b>	<b>NumDF</b>	<b>DenDF</b>	<b>F</b>	<b>P</b>
Heatwave treatment (HW)	2	142.6	0.078	0.925
Urbanization predator	1	143.9	0.894	0.346
Urbanization prey	1	146.0	0.024	0.877
HW × Urbanization predator	2	143.6	0.244	0.784
HW × Urbanization prey	2	145.2	3.558	<b>0.031</b>
Urbanization predator × Urbanization prey	1	145.9	0.232	0.631
HW × Urbanization predator × Urbanization prey	2	146.3	5.090	<b>0.007</b>
Time point attack	2	296.4	7.007	<b>0.001</b>
Sex predator	1	148.6	0.413	0.521
Prey ( <i>Daphnia</i> ) size (mm)	1	130.2	0.835	0.363
Predator ( <i>Ischnura</i> ) body mass (mg, corrected)	1	146.4	1.110	0.294
<b>C. Predation rate (<math>N_{\text{eaten}}/N_{\text{initial}} \cdot h^{-1}</math>)</b>	<b>NumDF</b>	<b>DenDF</b>	<b>F</b>	<b>P</b>
Heatwave treatment (HW)	2	106.8	7.079	<b>0.001</b>
Urbanization predator	1	21.3	2.701	0.115
Urbanization prey	1	23.1	1.597	0.219
HW × Urbanization predator	2	105.3	1.101	0.336
HW × Urbanization prey	2	111.8	1.531	0.221
Urbanization predator × Urbanization prey	1	121.3	5.663	<b>0.019</b>
HW × Urbanization predator × Urbanization prey	2	112.5	0.277	0.758
Sex predator	1	139.8	1.149	0.286
Prey ( <i>Daphnia</i> ) size (mm)	1	140.1	11.151	<b>0.001</b>
Predator ( <i>Ischnura</i> ) body mass (wet weight, mg)	1	142.2	0.704	0.403

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716 **Figure legends**

717 **Figure 1.** Schematic representation of the experimental scheme for the predation trials. Both urban  
718 and rural *Ischnura* predators (left) and *Daphnia* prey (right) were reared at 20 and 24°C in the lab  
719 (rearing procedures are detailed in material and methods), and thereafter exposed to either one of  
720 three five-day simulated heatwave treatments (HW20-28°C, HW24-28°C, HW24-32°C),  
721 independently. On the final day of each simulated heatwave, one predator and 20 prey were combined  
722 in a predation trial during which functional response parameters (encounter rate, handling time,  
723 predation rate) were assessed. We tested all four possible combinations of predator and prey  
724 urbanization background at each of the two heatwave temperatures (each time matching predator and  
725 prey of the same HW treatment). For each predator background (urban/rural) × prey background  
726 (urban/rural) × heatwave treatment (HW20-28°C, HW24-28°C, HW24-32°C) combination 12  
727 replicate trials were conducted.

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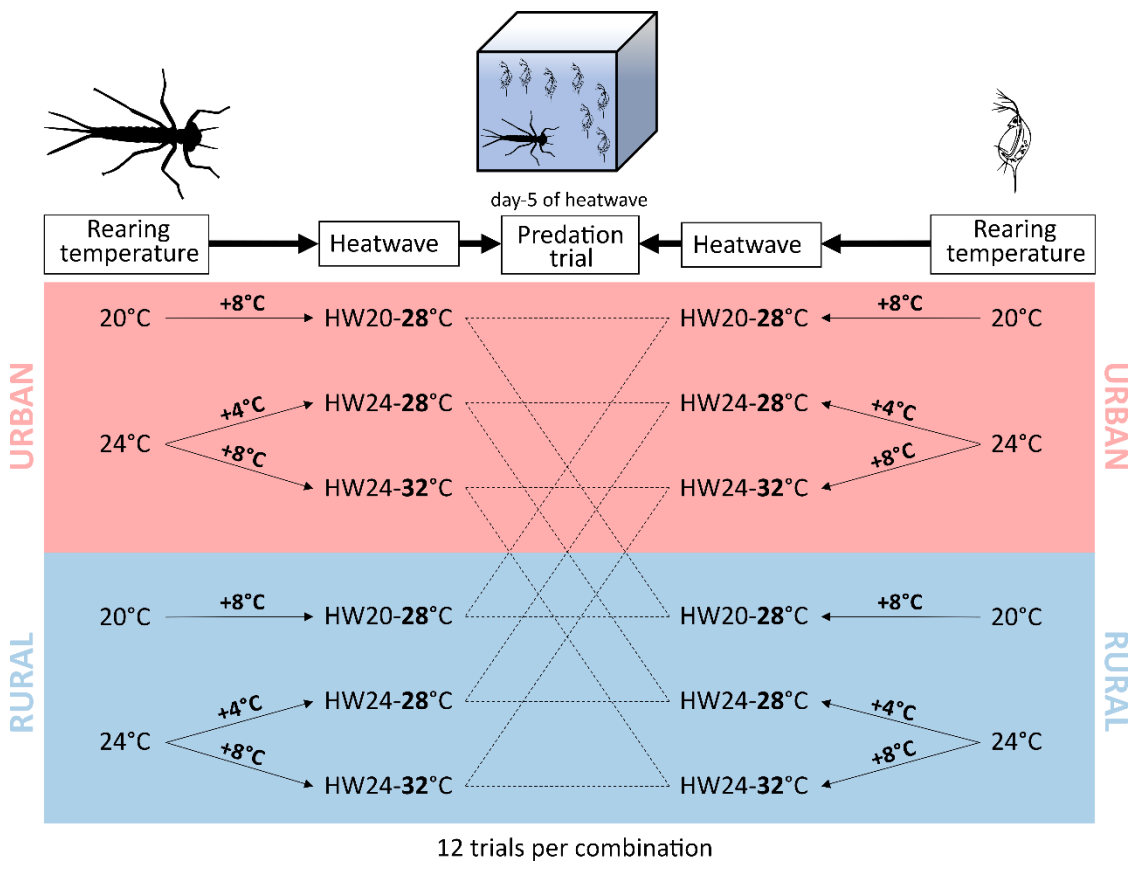
729 **Figure 2.** (A, B) Encounter rate ( $s^{-1}$ ), (D, E) handling time (s), and (G, H) predation rates (number of  
730 *Daphnia* eaten / number of initial *Daphnia*.  $h^{-1}$ ) of urban and rural nymphal *Ischnura elegans*  
731 predators on urban and rural *Daphnia magna* prey for three different heatwave scenarios. Panels A-B,  
732 D-E and G-H: x-axis labels urbanization background of predator, bar colour depicts urbanization  
733 background of prey (blue: rural; red: urban). Panels B, E, H show response variables averaged across  
734 the heatwave scenarios to visualize cryptic eco-evolutionary feedbacks. Shown are least squares  
735 means  $\pm 1$  SE. Panels C, F, I depict percentage change in each response variable relative to trials with  
736 a rural predator and a rural prey (predator urbanization on x-axis; dots reflect predation trials with  
737 rural prey, triangles indicate predation trials with urban prey; full arrows: change upon mismatch  
738 between urbanization level of predator and prey; dashed line: change from rural-rural to urban-urban).  
739 Red pictograms of predator and prey refer to an urban evolutionary background; blue pictograms refer  
740 to predators and prey with a rural background. Urban evolution in *Daphnia* buffers for the observed  
741 urban evolution-driven changes in an ecological response (encounter and predation rates) in the

742 predator (significant interaction between urbanization background of the predator × urbanization  
743 background of the prey, Table 1). Comparing encounter and predation rates of urban vs. rural  
744 predators using their respective prey type (horizontal lines, panels B, H) would have masked urban  
745 evo-to-eco feedbacks (i.e. ‘cryptic eco-evo feedback’). Shown are least squares means  $\pm 1$  SE.  
746 Different letters above (encounter rate, handling time, panels A,D) or within (predation rate, panel G)  
747 bars denote significant differences in response variables based on post-hoc comparisons reported on in  
748 results).

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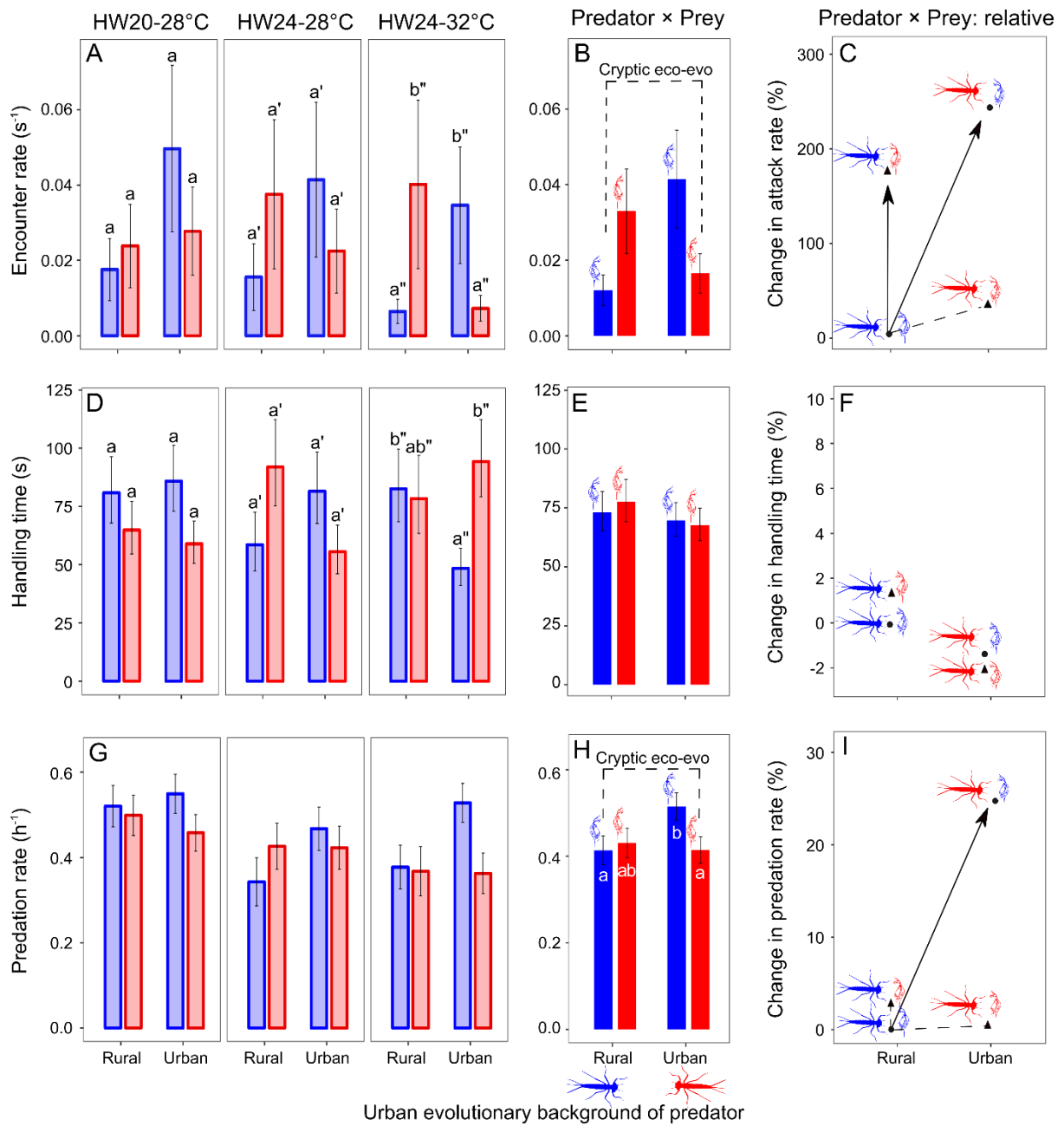
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Figure 1.





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Figure 2.