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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/ km², 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 2nd to 4th 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_{MAX} (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

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552 to the various drafts of the manuscript.

553

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

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556

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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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A. Encounter rate (s^{-1})	NumDF	DenDF	F	P
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 \times Urbanization predator	2	130.3	0.593	0.554
HW \times Urbanization prey	2	131.3	0.149	0.862
Urbanization predator \times Urbanization prey	1	131.6	16.111	<0.001
HW \times Urbanization predator \times Urbanization prey	2	134.3	2.675	0.073
Time point attack	1	146.0	5.536	0.020
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. Handling time (s)	NumDF	DenDF	F	P
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 \times Urbanization predator	2	143.6	0.244	0.784
HW \times Urbanization prey	2	145.2	3.558	0.031
Urbanization predator \times Urbanization prey	1	145.9	0.232	0.631
HW \times Urbanization predator \times Urbanization prey	2	146.3	5.090	0.007
Time point attack	2	296.4	7.007	0.001
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
C. Predation rate ($N_{\text{eaten}}/N_{\text{initial}} \cdot h^{-1}$)	NumDF	DenDF	F	P
Heatwave treatment (HW)	2	106.8	7.079	0.001
Urbanization predator	1	21.3	2.701	0.115
Urbanization prey	1	23.1	1.597	0.219
HW \times Urbanization predator	2	105.3	1.101	0.336
HW \times Urbanization prey	2	111.8	1.531	0.221
Urbanization predator \times Urbanization prey	1	121.3	5.663	0.019
HW \times Urbanization predator \times 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	0.001
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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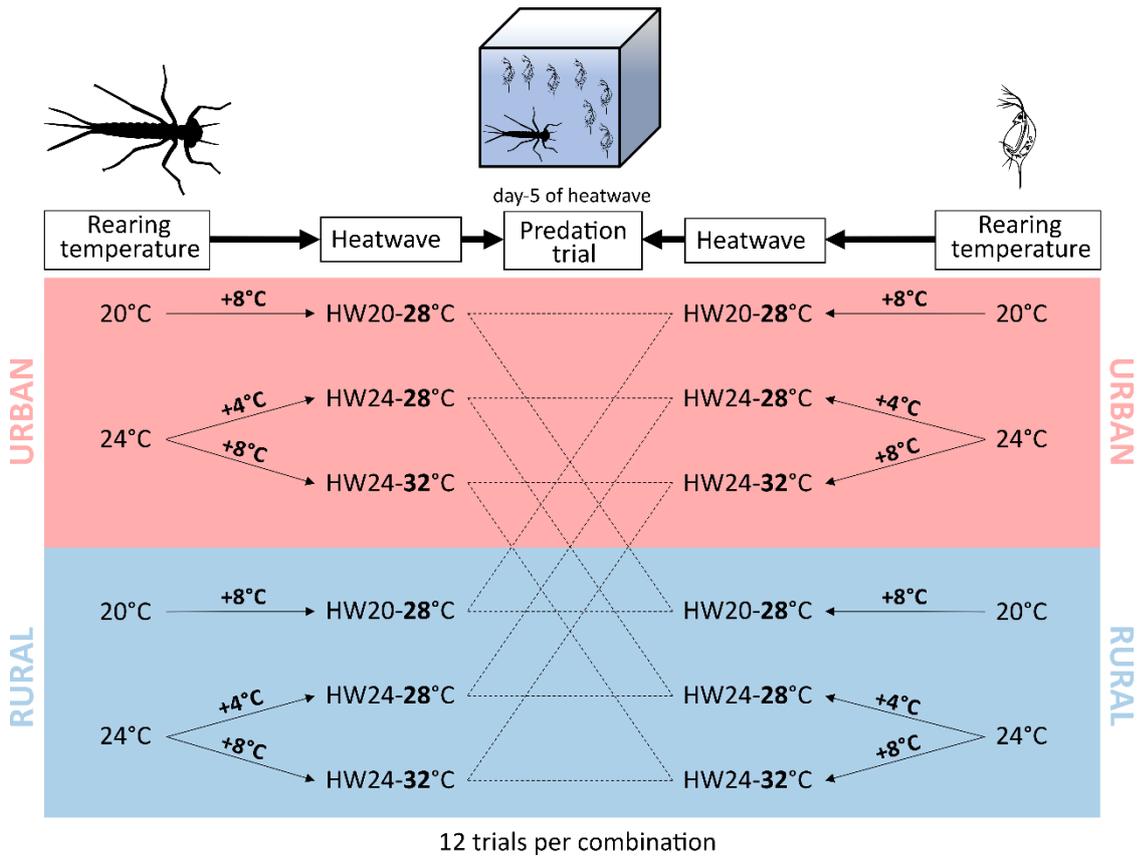
729 **Figure 2.** (A, B) Encounter rate (s^{-1}), (D, E) handling time (s), and (G, H) predation rates (number of
730 *Daphnia* eaten / number of initial *Daphnia*. h^{-1}) of urban and rural nymphal *Ischnura elegans*
731 predators on urban and rural *Daphnia magna* prey for three different heatwave 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 ± 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 ± 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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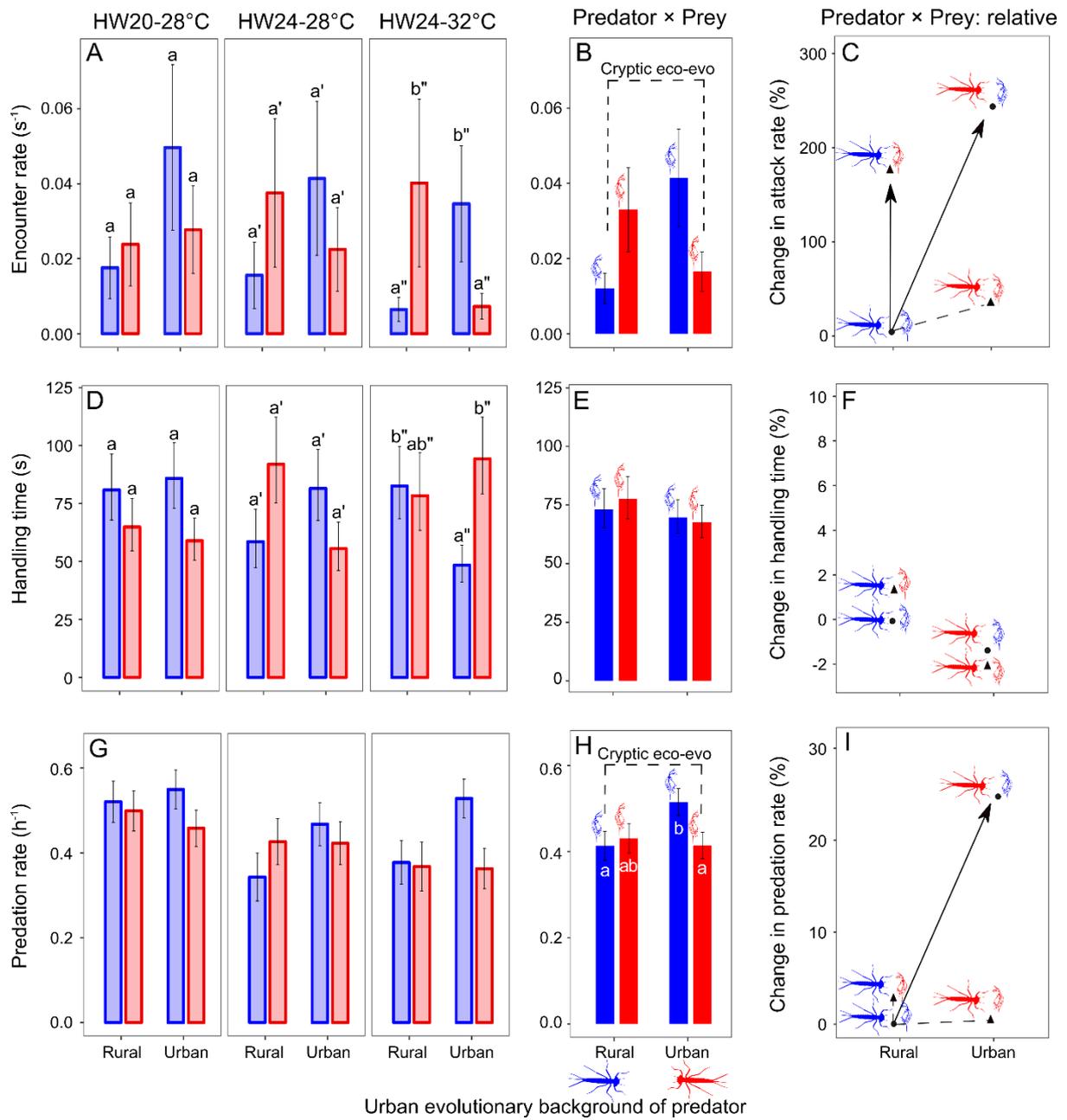


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Figure 1.



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Figure 2.