1 **Latitude-specific urbanization effects on life history traits in the damselfly *Ischnura***

2 ***elegans***

3

4

5

6 Gemma Palomar[1,2,†], Guillaume Wos[1,*,†], Robby Stoks[3], Szymon Sniegula[1,*]

7

8 [1] Institute of Nature Conservation Polish Academy of Sciences, al. Adama Mickiewicza 33,

9 31-120 Krakow, Poland

10 [2] Department of Genetics, Physiology, and Microbiology, Complutense University of Madrid,

11 C/Jose Antonio Novais 12, Madrid 28040, Spain

12 [3] Laboratory of Evolutionary Stress Ecology and Ecotoxicology, KU Leuven, Charles

13 Deberiotstraat 32, 3000 Leuven, Belgium

14

15 † Both authors contributed equally to this work

16

17 *Authors for correspondence

18 Guillaume Wos

19 wos@iop.krakow.pl

20 Szymon Sniegula

21 szymon.sniegula@gmail.com

22

23

24

25

26    **Abstract**

27    1. Many species are currently adapting to cities at different latitudes. Adaptation to

28      urbanization may require eco-evolutionary changes in response to temperature and

29      invasive species that may differ between latitudes.

30    2. Here, we studied single and combined effects of increased temperatures and invasive alien

31      predator presence on the phenotypic response of replicated urban and rural populations of

32      the damselfly *Ischnura elegans* and contrasted these between central and high latitudes.

33    3. Larvae were exposed to temperature treatments (current [20 ºC], mild warming [24 ºC],

34      and heat wave [28 ºC; for high latitude only]) crossed with the presence or absence of

35      chemical cues released by the spiny-cheek crayfish (*Faxonius limosus*), only present at the

36      central latitude. We measured treatment effects on larval development time, mass, and

37      growth rate.

38    4. Urbanization type affected all life history traits, yet these responses were often dependent

39      on latitude, temperature, and sex. Mild warming decreased mass in rural and increased

40      growth rate in urban populations. The effects of urbanization on mass were latitude-

41      dependent, with central-latitude populations having a greater phenotypic difference.

42      Urbanization effects were sex-specific with urban males being lighter and having a lower

43      growth rate than rural males. At the current temperature and mild warming, the predator

44      cue reduced the growth rate, and this independently of urbanization level and latitude of

45      origin. This pattern was reversed during a heat wave in high-latitude damselflies.

46    5. Our results highlight the context-dependency of evolutionary and plastic responses to

47      urbanisation, and caution for generalizing how populations respond to cities based on

48      populations at a single latitude.

49

50   Keywords: global warming, invasive alien species, *Ischnura elegans*, pace-of-life syndrome,

51   phenotypic plasticity, temperature, urbanization

52 **Introduction**

53 Urbanization has emerged as a strong and widespread source of selection affecting plant and

54 animal communities (Alberti, et al., 2017; Catullo et al., 2019). Urbanization is closely

55 related to drivers of eco-evolutionary change such as temperature and invasive species.

56 Within the cities, the dense concentration of pavement, buildings, and other surfaces that

57 absorb and retain heat creates 'urban heat islands' in which temperature is higher (Tam et al.,

58 2015). Urbanization may also favour the introduction of invasive species reducing diversity

59 by competitive exclusion of native species (McKinney, 2006). These phenomena together

60 with other factors related to urbanization, such as pollution or noise, create particular

61 environments within the cities that drive evolutionary changes in organisms, e.g. at the

62 phenotypic and physiological levels, and in their interactions with other organisms (Alberti,

63 et al., 2017; Lambert et al., 2021).

64 Global warming and latitudial gradient might further interact wtih urbanization and

65 alter organisms thermal plasticity and evolution (Verheyen et al. 2019). These plastic and

66 evolutionary responses may differ across the species latitudinal distribution because

67 populations from different latitudes encounter different seasonal time constraints (Stoks et al.,

68 2012). Since plastic responses are considered the first response to buffer the effects of novel

69 environmental stressors (Fox et al., 2019), investigating reaction norms of organisms from

70 urban and rural populations at different latitudes in response to warming and stress caused by

71 invasive alien predators may provide valuable insights to forecast species' phenotypic

72 response to the rapid local and global changes (Verheyen et al., 2019). Life history traits such

73 as body size are responsive to urbanization, although the trends are taxon dependent. While a

74 reduction in body size was observed in urban bumblebees (Eggenberger et al., 2019) and

75 birds (Meillère et al., 2015), the opposite pattern was found in moths (Merckx, et al., 2018).

76 Urbanization effects may also be sex-specific, e.g. urban males are bigger in butterflies

4

77    (Kaiser et al., 2016).    Available theories predict that when facing different ecological

78    conditions, as generally observed along an urbanization or latitudinal gradient, organisms

79    may be ranked from a slow- to fast-living continuum ('pace-of-life' syndrome; Brans & De

80    Meester, 2018; Réale et al., 2010). This may be reflected by a shift towards a faster pace-of-

81    life with accelerated growth, rapid development, and lower body mass as urbanization

82    increases (Brans & De Meester, 2018; Debecker et al., 2016). Therefore, as urbanization is

83    expected to affect life history strategies and patterns of covariation between traits, it is

84    important to consider multiple life history traits, both sexes and other interacting drivers of

85    evolutionary change such latitude-specific growth season length and warming, to better

86    reflect anthropogenic impacts of cities on organisms.

87         In this study, we tested and contrasted the response of life history traits to single and

88    combined thermal and invasive alien predator treatments between urban and rural populations

89    from different latitudes. Special attention went to whether these life history responses were

90    sex specific. We ran a common garden experiment on the damselfly *Ischnura elegans* from

91    replicated rural and urban populations at high- (southern Sweden) and central- (southern

92    Poland) latitudes. In damselflies, urban populations were shown to grow slower than rural

93    populations at central latitudes (Tüzün & Stoks, 2021), but whether such pattern is consistent

94    across the species latitudinal range requires further investigation. At the central latitude, the

95    higher annual temperatures and associated longer growth season allow more generations per

96    year, but less time per generation, thereby causing higher seasonal time constraints (Corbet et

97    al., 2006; Stoks et al., 2012). As the effect of urbanization on life history may differ between

98    mild warming and heat wave temperatures (e.g. 30 °C), we tested both in the same study.

99    Besides a thermal treatment (mild warming and a heat wave), we imposed a treatment where

100    we manipulated the presence of chemical predator cues from an invasive alien predator, the

101    spiny-cheek crayfish, *Faxonius limosus*, which has been co-occurring with Polish *I. elegans*

102  populations for several decades, but has not been yet reported in Sweden. The spread of

103  invasive crayfish predator has been largely mediated by human activities and urban areas

104  represent an attractive environment for this species (Reynolds & Souty-Grosset, 2011).

105

106  **Materials and Methods**

107  *Study species*

108  *Ischnura elegans* is a common damselfly in Europe (Dijkstra & Schröter, 2020). Across a

109  latitudinal gradient, populations are characterized by different number of generations per year

110  (voltinism). At central latitudes (including Poland), populations are generally uni- and

111  bivoltine, meaning one or two generations per year, respectively (Corbet et al., 2006; Norling,

112  2021). At high latitudes (including Sweden), populations are usually uni- and semivoltine,

113  meaning that one or two years are required for completing one generation, respectively.

114

115  *Study populations*

116  Adult *I. elegans* females were collected from two urban and two rural ponds each in southern

117  Sweden (hereafter, high latitude) and in southern Poland (hereafter, central latitude) (Fig. 1,

118  Table S1) on 22-23 June 2021, as described in Sniegula et al. (2020). The distances between

119  urban and rural sites ranged from 5 to 19 km at the high latitude and from 26 to 96 km at the

120  central latitude. We quantified the level of urbanization based on the percentage of

121  impervious surface from the high-resolution layer database (20 m resolution) (EEA, 2020).

122  We created a circular buffer of 1 km around each sampling location and calculated the

123  average value of imperviousness in each buffer using Quantum-GIS (QGIS Development

124  Team, 2017). Ponds were defined as urban when the average percentage of impervious

125  surface area within the buffer was above 20 % and as rural when the percentage was below

126  1.5 % (Brans & De Meester, 2018).

127    Water temperatures in the shallow parts of the collection ponds were estimated using

128  the Lake Model FLake (2009) that closely matches the actual temperature measured in situ

129  (Dinh Van et al., 2014). The modelled temperatures indicated minor differences in water

130  temperature among ponds within- and between both latitudes (Fig. S1A and B). As the FLake

131  model does not include impervious surface as a parameter and because water temperatures

132  within a pond might vary depending on various parameters such as depth or sun exposure, we

133  also placed temperature loggers (50 cm depth) in five ponds during summer and fall, and in

134  one pond during an entire year (2021 and/or 2022; Fig. S1C and D). Based on FLake and

135  dataloggers estimates, the average temperature in central- and high latitude ponds and

136  between urban and rural ponds were similar and oscillated around 20 °C and were below 24

137  °C during summer months (except one record in one Polish pond, Fig. 1C). Based on this and

138  on previous records of freshwater summer water temperatures at these latitudes (Debecker &

139  Stoks, 2019; Dinh Van et al., 2014), we set the following experimental temperatures: 20 °C

140  corresponding to the current mean summer water temperature, 24 °C corresponding to the

141  predicted increased temperature by 2100 under SSP8.5 scenario (Masson-Delmotte et al.,

142  2021), and 28 °C matching a simulated heat wave.

143

144  *The crayfish predator*

145  The spiny-cheek crayfish *Faxonius limosus* is an invasive alien predator and an active

146  colonizer that has locally co-occurred with the central-latitude *I. elegans* for at least 50 years

147  but has not been reported in Scandinavia (Artportalen, 2022; Commission Implementing

148  Regulation, 2016; Kouba et al., 2014). The spiny-cheek crayfish is widely reported in Poland

149  in rural and urban areas (The General Directorate for Environmental Protection, 2018). The

150  spread of the spiny-cheek crayfish is driven by human activities (aquaculture, trading), and

151  natural spread in Europe (Reynolds & Souty-Grosset, 2011) and occurs frequently in warm

152    temperatures (up to 25 ºC) and in polluted waters (Chucholl, 2016). Potential future invasions

153    are more likely to occur in urban areas. Prior the experiment, *F. limosus* were collected from

154    Kryspinów Lake in southern Poland (50°3'0.461"N, 19°47'20.85"E) and transported to the

155    Institute of Nature Conservation of the Polish Academy of Sciences, Kraków, Poland (INC

156    PAS). Three crayfishes were kept in an aquarium holding 50 L of dechlorinated tap water

157    along with a control aquarium with 50 L of dechlorinated tap water. Crayfish collection and

158    housing were done with permission from the Regional Directorate for Environmental

159    Protection in Kraków (ref. OP.672.4.2021.GZ).

160

161    *Housing and temperature treatment*

162    Adult female damselflies were individually placed in plastic cups with perforated lids and

163    wet filter paper for egg laying. Females were kept at ca. 22°C and natural daylight

164    (photoperiod). In total 80 clutches (10 clutches per population, hereafter families) were used

165    in the experiment. The design of the experiment involved the same pre-winter and winter

166    rearing conditions (hereafter, first part of the experiment) for all larvae. During post-winter

167    rearing conditions (hereafter, second part of the experiment), larvae were exposed to different

168    thermal treatments and, on the day of entering into the last instar prior emergence (F-0),

169    larvae were exposed to the predator cue treatments for the next 5 days. The experiment ended

170    at the end of this 5-day predator cue exposure period.

171          The first part of the experiment was carried out in plastic containers (size 22×16 cm,

172    height 11 cm) filled with 1500 mL of dechlorinated tap water. Containers were kept in an

173    incubator at 22 °C and a photoperiod of L:D 20:4h. In these containers, larvae were reared in

174    groups. The photoperiod indicated the longest day length at the high latitude collection site,

175    and was expected to create high development and growth rates in both studied latitudes,

176    especially during post-winter conditions (Norling, 2021). Each container was supplied with a

177   plastic structure to minimise predation among larvae. Since there were six different

178   treatments after winter (i.e. during the second part of the experiment), larvae from each of the

179   eight sampled populations were divided into six containers. At hatching, four larvae from

180   each family were randomly placed in each of the six containers, totalling 40 larvae per

181   container (4 larvae x 10 families). This approach ensured that each container started with

182   exactly the same number of individuals from each family. Larvae were fed *ad libitum* with

183   laboratory-cultured *Artemia nauplii*, twice a day on week days and once a day on weekend

184   days. Furthermore, after three weeks of growing, we supplemented the feeding with live

185   *Daphnia* sp. two times a week until autumn conditions. The position of the containers was

186   randomized weekly within each incubator.

187       On 06 August 2021 (ca. four weeks after larvae had hatched), we started simulating

188   autumn temperatures and photoperiods (hereafter, thermo-photoperiod) and, three weeks

189   later, winter conditions. This procedure allowed larvae to experience a winter diapause, as it

190   occurs in nature (Corbet 2006; Norling 2021). With a weekly interval, we gradually reduced

191   the initial thermo-photoperiod from 22 °C and 20:4 h to 6 °C and 0:24 h L:D (simulated

192   winter conditions). A detailed description of rearing thermo-photoperiods during the entire

193   experiment are presented in Fig. S2. During the simulated winter, larvae were fed three times

194   a week with *Artemia nauplii*. On 22 November 2022, we started the second part of the rearing

195   experiment. We transferred the larvae to individual 200 mL cups (height = 9 cm, diameter =

196   4 cm) filled with 100 mL of dechlorinated water and placed each cup into an incubator at 10

197   °C and 4:20 h L:D. With a two-day interval, we gradually increased the thermo-photoperiod

198   to the respective thermal treatment: 20 °C (control), 24 °C (mild warming) or 28 °C (heat

199   wave, high latitude only) with the same 20:4 h L:D photoperiod for all temperature

200   treatments (Fig. S2). For logistic reasons we could not set the 28 °C temperature treatment

201   group for the central-latitude populations. We therefore split our design into two experiments:

9

202 Experiment 1 focusing on high- and central latitude populations raised at 20 °C and 24 °C,

203 and Experiment 2 focusing on high-latitude populations raised at 20 °C, 24 °C and 28 °C.

204 Throughout the second part of the experiment, larvae were fed daily with *Artemia*

205 *nauplii*. Because larvae came from different latitudes and because rearing temperature also

206 affects larval development rate, larvae reached the F-0 stage at different dates. This led to

207 larvae being exposed to the post-winter temperature treatment for different durations. For

208 each larva, we reported the exact time in days at the temperature treatment during the second

209 half of the experiment ('thermal exposure duration') and this time was included as a covariate

210 in the statistical models (except for developmental time). In addition, when larvae entered F-

211 0, we identified the sex of each individual to test for sex-specific responses to the treatments

212 (Central latitude N $_{females}$ = 73, N $_{males}$ = 68; High latitude N $_{females}$ = 158, N $_{males}$ = 182).

213

214 *Predator cue treatment*

215 When larvae entered the F-0 stage, we applied a five-day-long predator cue treatment. The

216 water level in each cup was reduced to 67 mL and refilled with 33 mL of water from the

217 crayfish aquarium (with predator cue) or the control aquarium (without predator cue). Cups

218 were refilled every second day to keep the predator cue approximately constant, considering

219 the length of predator cue biodegradation (Van Buskirk et al. 2014). Previous experiments

220 have demonstrated that predator cues affect damselfly life history traits, also in case of short-

221 time exposure (13 days exposure in Antoł & Sniegula, 2021; 3-9 days exposure in Van

222 Dievel et al., 2016).

223

224 *Response variables*

225 In total, 481 larvae survived and were phenotyped at the end of the experiment. Details on

226 sample size for each treatment combination and response variable for both experiments are

227   presented in Table S2. When larvae entered F-0 and before the application of the predator cue

228   treatment, we quantified three traits: development time (DT; number of days between

229   hatching and moult into F-0), wet mass (mass $_{F-0}$), and growth rate until F-0 (GR $_{F-0}$). Larval

230   wet mass was measured with an electronic balance (Radwag AS.62) and GR $_{F-0}$ was

231   calculated as ln(mass $_{F-0}$)/age $_{F-0}$. After the five-day exposure to a predator cue, we measured

232   the wet mass again (mass $_{final}$) and calculated the growth rate over the five-day period: GR$_{final}$

233   = [ln(mass $_{final}$) – ln(mass $_{F-0}$)]/5, as in McPeek et al. (2001) (Table S3).

234

235   *Statistical analyses*

236   All analyses were performed in R (R Core Team, 2013; RStudio Team, 2015). For univariate

237   statistics, we used generalized linear mixed-effects models (GLMMs). First, we ran a model

238   selection analysis (MuMin r package; Barton & Barton, 2015) to select the most appropriate

239   model for each phenotypic variable (DT, mass $_{F-0}$, GR $_{F-0,}$ and GR $_{final}$). We included in the

240   initial model the following predictors: latitude (only for experiment 1), sex, temperature,

241   urbanization, predator (for GR $_{final}$ only), and all the possible interactions; thermal exposure

242   duration was added as a covariate (except for DT because this variable was strongly

243   correlated with exposure duration) and population nested in latitude as a random factor. For

244   each phenotypic variable, model selection analysis was based on the corrected Akaike's

245   information criteria for sample size (AICc) and weights as criteria to determine the best

246   explanatory linear model by keeping only the most relevant predictors and interactions (Table

247   S4). In Experiment 1, we compared urbanization type (rural vs. urban population), latitude

248   (high- and central-latitude), temperature (20 ℃ and 24 ℃), sex (males and females) and

249   predator (for GR $_{final}$ only). We ran specific models for each variable: DT $_{F-0}$ with a Poisson

250   distribution, and mass $_{F-0}$, GR $_{F-0}$ and GR $_{Final}$ with Gaussian distributions, based on the output

251   of the model selection analysis. The variables were normally distributed. In Experiment 2, we

11

252    tested for effects of urbanisation type, temperature (20, 24 and 28°C) and predator (for GR

253    $_{final}$ only) in high-latitude populations. To fit the GLMMs, we used the function glmmTMB

254    (glmmTMB package; Magnusson et al., 2017). P-values were obtained using the Wald chi-

255    square test (Wald X²) implemented in the car package (Fox & Weisberg, 2015).

256        To analyse in detail the multivariate phenotypic responses of the larvae we applied

257    Phenotypic Trajectory Analysis (PTA) following the procedure and R scripts described in

258    Adams & Collyer (2009) and Collyer & Adams (2007). For this analysis, we used the

259    measurements when larvae entered F-0 (hence, prior to the predator cue treatment). We used

260    PTA to compare trajectories of high- and central-latitude populations in response to

261    urbanization (evolutionary change expressed as a magnitude of phenotypic difference

262    between rural and urban populations) and to temperature (plastic change expressed as the

263    slope of the thermal reaction norm). We ran PTA both with the sexes pooled and with males

264    and females separately. The procedure consists on a vector analysis in a multivariate space of

265    phenotypic trait change. First, we conducted a PCA using DT $_{F-0}$, mass $_{F-0,}$ and GR $_{F-0}$. To

266    account for the fact that damselflies were exposed to the temperature treatment for different

267    durations, we ran a GLM model for mass $_{F-0}$ and GR $_{F-0}$ with 'thermal exposure duration' as

268    covariate and extracted the residuals that were subsequently used for the PCA and PTA

269    analyses. Then, we created vectors connecting centroids of groups of individuals to calculate

270    and compare the difference in length (magnitude) and angle (direction, θ) between groups.

271    Significance in magnitude and direction were estimated using a permutation procedure ($N =$

272    1000 permutations); significant differences in the magnitude and direction between vectors

273    were interpreted as different evolutionary or plastic trajectories.

274

275    **Results**

276    *Experiment 1*

*Univariate response patterns*

The results testing for the effects of the treatments on DT, mass, and GR are shown in Table 1. Urbanization had no main effect, but several interactions involving urbanization were significant. At 20 °C, urban larvae had a lower mass $_{F-0}$ and GR $_{F-0}$ compared to rural larvae. Mild warming (24 °C) reduced mass $_{F-0}$, especially in rural larvae, and increased GR $_{F-0}$, especially in urban larvae, eliminating the differences for these traits between urban and rural populations at 24 °C (interaction urbanization × temperature) (Fig. 2A and 2B). While urban and rural females did not differ in mass $_{F-0}$ and GR $_{F-0}$, urban males were lighter and showed a lower GR $_{F-0}$ than rural males (interaction urbanization × sex) (Fig. 2C and 2D). Urbanization decreased larval mass, but in central-latitude individuals only (interaction latitude × urbanization) (Fig. 1); there was a larger difference in mass $_{F-0}$ between latitudes in females than in males (interaction latitude × sex) (Fig. S3A). The interaction latitude × temperature was significant for GR $_{F-0}$, with higher values of GR $_{F-0}$ in central- compared to high latitude individuals but only at 24 °C (Fig. S3B). DT was not affected by urbanization type, but independently affected by latitude and temperature, with a shorter development time in central-latitude larvae and in larvae reared at 24 °C (Table S5; Fig. S4).

During the five-day predator cue treatment, latitude and predator treatment had significant effect on GR $_{final}$, with faster growth rate in central-latitude larvae and in the absence of predator cues (Table S5; Fig. S5). None of the interaction terms were significant for the GR $_{final.}$

*Phenotypic Trajectory Analysis*

For central-latitude populations, evolutionary changes in response to urbanization between larvae raised at 20 °C and 24 °C did not differ in length (Var$_{length}$ = 0.39, $p$ = 0.306) nor in direction (θ = 10.2°, $p$ = 0.830). Evolutionary changes driven by urbanization pointed mostly

13

302    to a lower mass and growth rate in urban areas (Fig. 3A). Plastic changes in response to mild

303    warming temperature between rural and urban populations did not differ in length ($Var_{length}$ =

304    0.27, $p$ = 0.300) nor in direction (θ = 15.1°, $p$ = 0.537). Urban and rural populations

305    responded in a similar way to mild warming temperature with a shorter development time

306    when temperature increased (Fig. 3C).

307        Next, we ran similar analyses for males and females separately. For males, we found

308    the same patterns for evolutionary and plastic changes as with the sexes pooled (Fig. S6A and

309    C). For females, we found different evolutionary trajectories (differences in direction only)

310    between females raised at 20 °C and 24 °C (Fig. S7A); at 20 °C evolutionary changes in

311    response to urbanization pointed to a decrease in mass and GR $_{F-0}$ and the pattern was

312    reversed at 24 °C. Thermal plasticity did not differ between rural and urban females (Fig.

313    S7C).

314        For high-latitude populations, when sexes were pooled, evolutionary changes in

315    response to urbanization differed both in length ($Var_{length}$ = 0.70, $p$ = 0.007) and direction (θ =

316    132.4°, $p$ = 0.001) between the two temperature treatments (Fig. 3B). Urban larvae had a

317    longer DT, and lower mass and GR than rural larvae at 20 °C, whereas there was little

318    difference between urban and rural damselflies at 24 °C. Plastic changes in response to an

319    increase in temperature showed no difference in magnitude between rural and urban

320    populations ($Var_{length}$ = 0.26, $p$ = 0.296) but significant differences in direction (θ = 39.9°, p =

321    0.009) with a decrease in mass and GR at 24 °C only being present in rural populations (Fig.

322    3D).

323        When looking at each sex separately, for males, we found the exact same patterns for

324    evolutionary (Fig. S6B) and plastic (Fig. S6D) changes as with the sexes pooled. For females,

325    evolutionary changes differed only in direction between the temperature treatments; at 20 °C

326    evolutionary changes pointed to an increase in development time in urban area and at 24 °C

14

327  to an increase in mass and GR $_{F-0}$ (Fig S7B). Plastic changes to mild warming temperature for

328  females followed the same pattern as with the sexes pooled (Fig. S7D).

329

330  ***Experiment 2.***

331  *Univariate response patterns*

332  In the set of high-latitude urban and rural larvae tested at the three temperatures, the score

333  life-history traits (mass $_{F0}$, DT $_{F0,}$ and GR $_{F0}$) were not affected by urbanization, but were

334  affected by temperature and sex (Table S4). The interaction temperature $\times$ sex was significant

335  for both mass $_{F-0}$ and GR $_{F-0}$, with higher values in mass $_{F-0}$ and GR $_{F-0}$ in females, especially

336  at 24 °C (Fig. S8). GR $_{final}$ was not affected by urbanization. Exposure to the predator cue

337  during the five-day-long treatment reduced GR $_{final}$ at 20°C and 24°C, but instead increased

338  GR $_{final}$ at 28°C (predator $\times$ temperature) (Fig. 1).

339

340  *Phenotypic Trajectory Analysis*

341  Phenotypic trajectory analysis showed that high-latitude populations exhibited different

342  evolutionary changes in response to urbanization across the three temperature treatments

343  (sexes pooled). We did not find significant variation in magnitude between the three vectors

344  (Var$_{length}$ = 0.02, $p$ = 0.218) but we found significant differences in their direction (Var$_{direction}$

345  = 3547.0, $p$ = 0.001) (Fig. 4A). At 20 °C and 28°C, urbanization is mostly accompanied by

346  longer development and lower mass $_{F-0}$ and GR $_{F-0,}$ whereas at 24 °C differences between

347  rural and urban individuals were rather small. For plastic changes, PTA revealed significant

348  differences in length (Var$_{length}$ = 0.720, $p$ = 0.016) but not in direction (Var$_{direction}$ = 10.0, $p$ =

349  0.222) between the two vectors indicating a greater thermal plasticity in urban than in rural

350  populations (Fig. 4B).

15

351     When looking at each sex separately, evolutionary changes in response to

352     urbanization were similar for males and females and followed the same patterns as with the

353     sexes pooled (Fig. S9A and B). For plastic changes, no difference in both length and direction

354     was found in males (Fig. S9C), whereas differences in length were found in females (Fig.

355     S9D); with a greater thermal plastic response in females from urban than rural populations.

356

357     **Discussion**

358     Here, we studied phenotypic adaptation in an ectotherm considering several life-history traits

359     and interacting drivers of evolutionary change. Our results pointed to effects of urbanization

360     in combination with temperature on mass and GR. Notably, the direction and magnitude of

361     the evolutionary and plastic responses to temperature in life history traits (i.e. body mass and

362     GR) were not uniform across studied latitudes. The response to a predator was rather

363     independent of the latitudinal origin of the populations and of the urbanization type. Our

364     results shed some light on the effect of interacting anthropogenic factors in ectotherms life-

365     history traits and how these patterns are latitude dependent.

366

367     *Effects on individual traits*

368     In Experiment 1, we found negative effects of urbanization on larval growth rate and mass,

369     but these effects differed between latitudes and depended on temperature. This partially

370     confirmed that urbanization can decrease mass in insects (Merckx, et al., 2018), and that mass

371     decrease is temperature-dependent (Diamond et al., 2014). A mild warming temperature was

372     sufficient to remove the differences between rural and urban larval mass and growth rate.

373     Intriguingly, these two traits showed higher thermal plasticity at different urbanization types.

374     While mass showed steeper thermal reaction norm in rural populations, urban populations

375     were more plastic for GR. We might hypothesise that the effect of temperature was stronger

16

376     than the effect of urbanization and that growth rate reached some physiological limits at 24

377     °C (growth rate did not increase at 28 °C in high latitude populations), however, the pattern

378     was less clear for the mass.

379          Notably, the effects of urbanization were also latitude-dependent. The latitudinal

380     difference may be attributed to different life-history strategies related to voltinism and

381     seasonal time constraints. At central latitudes, the species produces, on average, one

382     generation per year more than at high latitudes (Corbet et al., 2006; Norling, 2021).

383     Therefore, central-latitude larvae are likely more time constrained: they develop within a

384     shorter time, grow faster and reach lower mass than at high latitudes (as seen for the species

385     between low and high latitudes, e.g. Debecker and Stoks 2019). A higher growth rate indeed

386     often comes at a cost of having a smaller mass at emergence (Sniegula et al., 2018). In

387     current study, latitudinal differences in mass seemed to be amplified by urbanization. Indeed,

388     with climate change, there might be a further increase in time constraints in urban areas for

389     central-latitude individuals that might arose from the heat-island effects (Chick et al., 2019).

390     However, based on our field measurements, contemporary water temperatures in urban and

391     rural ponds differed slightly (Fig. S1), and it is difficult to predict whether climate change

392     will increase this difference in temperature.

393          Independently of urbanization and latitude, the predator cue negatively affected larval

394     GR. In insects, exposure to a predator alters metabolism (Cinel et al., 2020) and foraging

395     activity (Kohler & McPeek, 1989), with downstream effects on larval development and

396     growth rate. Our results indicate that both central- and high-latitude populations reacted in a

397     similar way to the predator cue in the traits we measured. These results contrast with a recent

398     study showing a differential response in development time in *I. elegans* eggs when exposed

399     to cues from non-native invasive predators (spiny-cheek and signal crayfish *Pacifastacus*

400     *leniusculus*) (Antoł & Sniegula, 2021). However, an exposure to a phylogenetically related

17

401    predator species, i.e. noble crayfish (*Astacus astacus*) present at both latitudes (Kouba et al.,

402    2014), might enable predator cue recognition and trigger similar responses between the two

403    latitudes (Anton et al., 2020).

404        Results from Experiment 2 did not provide evidence that urban and rural populations

405    coped differently with a simulated heat wave, as previously demonstrated in damselflies

406    (Tüzün & Stoks, 2021) and other ectotherms (Brans et al., 2017; Campbell-Staton et al.,

407    2020). This may stem from the minor mean temperature differences between urban and rural

408    ponds at our study sites or different experimental approaches. Yet, we found a significant

409    effect of temperature in combination with predator cues. Damselflies grew faster in the

410    absence of a predator cue, but only in current and warming temperature. Interestingly,

411    exposure to the predator cues increased larval growth under a heat wave treatment. A

412    combination of stressors may change a strategy in potential prey to escape predator exposure

413    (Warkentin, 2011). Here, an accelerated larval growth in the presence of a predator cue under

414    heat wave may be part of an escape strategy to reduce the time of exposure to predators, as

415    previously shown in odonate species (Antoł & Sniegula, 2021; Stoks et al., 2012) and other

416    taxa (Chivers et al., 2001).

417        In both experiments, we found multiple sex-specific effects on mass and GR with

418    greater phenotypic differences between urbanization types and temperatures in males. These

419    results supported previous studies in which sex-specific effects were found in ectotherms

420    coping with various stressors, i.e. urbanization (Kaiser et al., 2016) and heat stress (Sniegula

421    et al., 2017). Sex-specific effects are generally more pronounced in species with strong sexual

422    dimorphism which is the case in damselflies, with females being usually larger and heavier

423    than males (Corbet, 1999). Sex-specific effects are also common in protandrous species with

424    a strong selection acting on males to emerge before females in order to maximise their mating

425    opportunities (Badyaev, 2002). Hence, different selective pressures resulting in different life-

18

426    history strategies and associated resource allocation patterns between males and females may

427    lead to different types of trade-offs. For instance, female butterflies maintained a relative high

428    body mass under different thermal conditions compared to males because mass was more

429    important for females in reproduction than for males (Fischer & Fiedler, 2000). This is likely

430    to be the case in odonates (Sokolovska et al., 2000) and matched our observations of mass of

431    females being less affected by urbanization and temperature than males (at least until 24 °C;

432    Fig. S3A).

433

434    *Multivariate approach*

435    Our results indicate multivariate evolutionary change associated to urbanization at the high

436    latitude since larvae from urban and rural sites differed in life history traits and in their

437    thermal plasticity. In contrast, central-latitude damselflies exhibited similar direction and

438    magnitude of evolutionary trajectories in response to urbanization in the two temperature

439    treatments (Experiment 1). Similar results were found in high-latitude individuals in response

440    to urbanization and additional simulation of heat wave (Experiment 2). The 'pace-of-life'

441    syndrome predicts a shift towards a fast-living strategy when urbanization increases or

442    towards lower latitudes, which was supported empirically in birds and invertebrates (Brans &

443    De Meester, 2018; Charmantier et al., 2017; Debecker et al., 2016). We observed that only

444    high latitude damselflies expressed a consistent decrease in mass and growth rate associated

445    with urbanization at 20 ºC, which was further accompanied with an increase of development

446    time. Hence, we found no support for a faster pace-of-life in the studied populations. One

447    explanation might be the minor difference in water temperatures recorded in ponds from

448    which rural and urban *I. elegans* were collected. However, in the damselfly *Coenagrion*

449    *puella* a lower growth rate in urban compared to rural populations was also shown, despite

450    water temperatures being up to 3.5 °C higher in urban than in rural ponds (Tüzün & Stoks,

19

451 2021). In that study, the population-specific growth pattern was explained by relatively lower

452 temperature and hence shorter growing seasons in rural populations, i.e. compensation to

453 time constraints by increased growth rate. Our results might, therefore, reflect selection

454 caused by other sources of disturbance associated with the urban environment, e.g. different

455 concentrations of pollutants. Notably, we demonstrated sex-specific evolutionary trajectories

456 to urbanization at both latitudes. Therefore, the population origin (latitude) and the sex, which

457 entail different life-history strategies or trade-offs, were more likely to trigger different

458 trajectories than a shared response to urbanization.

459 Increased levels of environmental disturbance, including stress linked to suboptimal

460 temperatures, in cities is expected to favour phenotypic plasticity (Alberti, et al., 2017). In

461 contrast, rural and urban populations from the central latitude responded with similar

462 magnitude and direction to mild warming by decreasing developmental time, whereas high-

463 latitude populations showed similar plasticity in this trait to mild warming in terms of

464 magnitude, but not in terms of direction. However, in response to the heat wave, we did find

465 a greater magnitude of the plastic response in urban populations for $GR_{F-0}$ and mass,

466 matching the expectation of an increased phenotypic plasticity in urban populations. Different

467 effects of urbanization on plasticity between the two latitudes may be partially caused by

468 different life history strategies: lower latitude organisms tend to have shorter generation times

469 (Corbet et al., 2006). Based on these results, we may infer that not only 'urban heat island'

470 effects impose differential selective pressures on organisms across urbanisation gradients

471 (Shochat et al., 2006), but also geographic origin and temperature exposition in rural and

472 urban populations have the potential to adjust the damselfly phenotype.

473

474 **Conclusion**

20

475    Despite the accumulating evidence that urbanization shapes phenotypes, and the expectation

476    these evolutionary and plastic responses may differ among latitudes, the latter has rarely been

477    tested. We showed that the damselfly responses to urbanization differed between latitudes.

478    Notably, the latitude-specific responses to urbanisation were temperature-dependent, which

479    could be explained by differences in life-history strategies across latitudes. Both urban and

480    rural populations had the potential to produce a plastic response to warming, yet the

481    magnitude and direction of the plastic changes differed between latitudes. Moreover, we

482    added to the knowledge that the response to urbanization can differ between sexes. In

483    contrast, urban and rural damselflies responded similarly to the presence of a predator cue,

484    the later interacting with different temperatures. Our results highlight the context-dependency

485    of evolutionary and plastic responses to urbanisation, and caution for generalizing of how

486    populations respond to cities based on populations at a single latitude.

487

488

**Reference**

Adams, D. C., & Collyer, M. L. (2009). A general framework for the analysis of phenotypic trajectories in evolutionary studies. *Evolution: International Journal of Organic Evolution*, *63*(5), 1143–1154.

Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., Hunt, V. M., Apgar, T. M., & Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Sciences*, *114*(34), 8951–8956. https://doi.org/10.1073/pnas.1606034114

Alberti, M., Marzluff, J., & Hunt, V. M. (2017). Urban driven phenotypic changes: Empirical observations and theoretical implications for eco-evolutionary feedback. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1712), 20160029.

Antoł, A., & Sniegula, S. (2021). Damselfly eggs alter their development rate in the presence of an invasive alien cue but not a native predator cue. *Ecology and Evolution*, *11*(14), 9361–9369. https://doi.org/10.1002/ece3.7729

Anton, A., Geraldi, N. R., Ricciardi, A., & Dick, J. T. A. (2020). Global determinants of prey naiveté to exotic predators. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1928), 20192978. https://doi.org/10.1098/rspb.2019.2978

Artportalen (Swedish Species Observation System) (2022). ArtDatabanken. dataset/occurrence. http://www.gbif.se/ipt/resource?r=artdata

Badyaev, A. V. (2002). Growing apart: An ontogenetic perspective on the evolution of sexual size dimorphism. *Trends in Ecology & Evolution*, *17*(8), 369–378. https://doi.org/10.1016/S0169-5347(02)02569-7

Barton, K., & Barton, M. K. (2015). Package 'mumin.' *Version*, *1*(18), 439.

Brans, K. I., & De Meester, L. (2018). City life on fast lanes: Urbanization induces an evolutionary shift towards a faster lifestyle in the water flea *Daphnia*. *Functional Ecology*, *32*(9), 2225–2240. https://doi.org/10.1111/1365-2435.13184

Brans, K. I., Jansen, M., Vanoverbeke, J., Tüzün, N., Stoks, R., & De Meester, L. (2017). The heat is on: Genetic adaptation to urbanization mediated by thermal tolerance and body size. *Global Change Biology*, *23*(12), 5218–5227. https://doi.org/10.1111/gcb.13784

Buskirk, J. V., Krügel, A., Kunz, J., Miss, F., & Stamm, A. (2014). The Rate of Degradation of Chemical Cues Indicating Predation Risk: An Experiment and Review. *Ethology*, *120*(9), 942–949. https://doi.org/10.1111/eth.12266

Campbell-Staton, S. C., Winchell, K. M., Rochette, N. C., Fredette, J., Maayan, I., Schweizer, R. M., & Catchen, J. (2020). Parallel selection on thermal physiology facilitates repeated adaptation of city lizards to urban heat islands. *Nature Ecology & Evolution*, *4*(4), Article 4. https://doi.org/10.1038/s41559-020-1131-8

Catullo, R. A., Llewelyn, J., Phillips, B. L., & Moritz, C. C. (2019). The potential for rapid evolution under anthropogenic climate change. *Current Biology*, *29*(19), R996–R1007. https://doi.org/10.1016/j.cub.2019.08.028

Charmantier, A., Demeyrier, V., Lambrechts, M., Perret, S., & Grégoire, A. (2017). Urbanization is associated with divergence in pace-of-life in great tits. *Frontiers in Ecology and Evolution*, *5*. https://www.frontiersin.org/articles/10.3389/fevo.2017.00053

Chick, L. D., Strickler, S. A., Perez, A., Martin, R. A., & Diamond, S. E. (2019). Urban heat islands advance the timing of reproduction in a social insect. *Journal of Thermal Biology*, *80*, 119–125. https://doi.org/10.1016/j.jtherbio.2019.01.004

Chivers, D. P., Kiesecker, J. M., Marco, A., Devito, J., Anderson, M. T., & Blaustein, A. R. (2001). Predator-induced life history changes in amphibians: Egg predation induces hatching. *Oikos*, *92*(1), 135–142. https://doi.org/10.1034/j.1600-0706.2001.920116.x

539    Chucholl, C. (2016). The bad and the super-bad: Prioritising the threat of six invasive alien to
540          three imperilled native crayfishes. *Biological Invasions*, *18*(7), 1967–1988.
541          https://doi.org/10.1007/s10530-016-1141-2
542    Cinel, S. D., Hahn, D. A., & Kawahara, A. Y. (2020). Predator-induced stress responses in
543          insects: A review. *Journal of Insect Physiology*, *122*, 104039.
544          https://doi.org/10.1016/j.jinsphys.2020.104039
545    Collyer, M. L., & Adams, D. C. (2007). Analysis of two-state multivariate phenotypic change
546          in ecological studies. *Ecology*, *88*(3), 683–692. https://doi.org/10.1890/06-0727
547    Commission Implementing Regulation. (2016). Regulation. 2016/1141 of 13 July 2016
548          adopting a list of invasive alien species of Union concern pursuant to Regulation (EU)
549          No 1143/2014 of the European Parliament and of the Council
550    Corbet, P. S. (1999). *Dragonflies: Behaviour and ecology of Odonata.* Harley books.
551    Corbet, P. S., Suhling, F., & Soendgerath, D. (2006). Voltinism of Odonata: A review.
552          *International Journal of Odonatology*, *9*(1), 1–44.
553    Debecker, S., Sanmartín-Villar, I., de Guinea-Luengo, M., Cordero-Rivera, A., & Stoks, R.
554          (2016). Integrating the pace-of-life syndrome across species, sexes and individuals:
555          Covariation of life history and personality under pesticide exposure. *Journal of
556          Animal Ecology*, *85*(3), 726–738. https://doi.org/10.1111/1365-2656.12499
557    Debecker, S., & Stoks, R. (2019). Pace of life syndrome under warming and pollution:
558          Integrating life history, behavior, and physiology across latitudes. *Ecological
559          Monographs*, *89*(1), e01332.
560    Diamond, S. E., Cayton, H., Wepprich, T., Jenkins, C. N., Dunn, R. R., Haddad, N. M., &
561          Ries, L. (2014). Unexpected phenological responses of butterflies to the interaction of
562          urbanization and geographic temperature. *Ecology*, *95*(9), 2613–2621.
563    Dijkstra, K.-D., & Schröter, A. (2020). *Field guide to the dragonflies of Britain and Europe*.
564          Bloomsbury Publishing.
565    Dinh Van, K., Janssens, L., Debecker, S., & Stoks, R. (2014). Temperature⬚and
566          latitude⬚specific individual growth rates shape the vulnerability of damselfly larvae
567          to a widespread pesticide. *Journal of Applied Ecology*, *51*(4), 919–928.
568    EEA. (2020). © European Union, Copernicus Land Monitoring Service [Accessed
569          17.02.2023]  https://land.copernicus.eu/pan-european.
570    Eggenberger, H., Frey, D., Pellissier, L., Ghazoul, J., Fontana, S., & Moretti, M. (2019).
571          Urban bumblebees are smaller and more phenotypically diverse than their rural
572          counterparts. *Journal of Animal Ecology*, *88*(10), 1522–1533.
573          https://doi.org/10.1111/1365-2656.13051
574    Fischer, K., & Fiedler, K. (2000). Sex-related differences in reaction norms in the butterfly
575          *Lycaena tityrus* (Lepidoptera: Lycaenidae). *Oikos*, *90*(2), 372–380.
576          https://doi.org/10.1034/j.1600-0706.2000.900218.x
577    Fox, J., & Weisberg, S. (2015). *Using car functions in other functions*.
578    Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitán-Espitia, J. D. (2019). Beyond
579          buying time: The role of plasticity in phenotypic adaptation to rapid environmental
580          change. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
581          *374*(1768), 20180174. https://doi.org/10.1098/rstb.2018.0174
582    Kaiser, A., Merckx, T., & Van Dyck, H. (2016). The Urban Heat Island and its spatial scale
583          dependent impact on survival and development in butterflies of different thermal
584          sensitivity. *Ecology and Evolution*, *6*(12), 4129–4140.
585          https://doi.org/10.1002/ece3.2166
586    Kohler, S. L., & McPeek, M. A. (1989). Predation risk and the foraging behavior of
587          competing stream insects. *Ecology*, *70*(6), 1811–1825.

588    Kouba, A., Petrusek, A., & Kozak, P. (2014). Continental-wide distribution of crayfish
589         species in Europe: Update and maps. *Knowledge and Management of Aquatic*
590         *Ecosystems*, *413*, 05. https://doi.org/10.1051/kmae/2014007
591    Lake Model Flake. (2009). *FLake*. Http://Www.Cosmo-
592         Model.Org/Content/Model/Modules/Flake/  [Accessed: 2-05-2017].
593    Lambert, M. R., Brans, K. I., Des Roches, S., Donihue, C. M., & Diamond, S. E. (2021).
594         Adaptive evolution in cities: Progress and misconceptions. *Trends in Ecology &*
595         *Evolution*, *36*(3), 239–257. https://doi.org/10.1016/j.tree.2020.11.002
596    Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., van
597         Bentham, K., Bolker, B., Brooks, M., & Brooks, M. M. (2017). Package
598         'glmmTMB.' *R Package Version 0.2. 0*.
599    Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S. L., Péan, C., Berger, S., Caud, N.,
600         Chen, Y., Goldfarb, L., & Gomis, M. I. (2021). Climate change 2021: The physical
601         science basis. *Contribution of Working Group I to the Sixth Assessment Report of the*
602         *Intergovernmental Panel on Climate Change*, 2.
603    McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological*
604         *Conservation*, *127*(3), 247–260. https://doi.org/10.1016/j.biocon.2005.09.005
605    McPeek, M. A., Grace, M., & Richardson, J. M. (2001). Physiological and behavioral
606         responses to predators shape the growth/predation risk trade☐off in damselflies.
607         *Ecology*, *82*(6), 1535–1545.
608    Meillère, A., Brischoux, F., Parenteau, C., & Angelier, F. (2015). Influence of urbanization
609         on body size, condition, and physiology in an urban exploiter: A multi-component
610         approach. *PloS One*, *10*(8), e0135685.
611    Merckx, T., Kaiser, A., & Van Dyck, H. (2018). Increased body size along urbanization
612         gradients at both community and intraspecific level in macro-moths. *Global Change*
613         *Biology*, *24*(8), 3837–3848. https://doi.org/10.1111/gcb.14151
614    Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L. F., Backeljau, T., Bonte, D., Brans, K. I.,
615         Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J. M. T., Fontaneto, D.,
616         Gianuca, A. T., Govaert, L., Hendrickx, F., Higuti, J., Lens, L., Martens, K., … Van
617         Dyck, H. (2018). Body-size shifts in aquatic and terrestrial urban communities.
618         *Nature*, *558*(7708), Article 7708. https://doi.org/10.1038/s41586-018-0140-0
619    Norling, U. (2021). Growth, winter preparations and timing of emergence in temperate zone
620         Odonata: Control by a succession of larval response patterns. *International Journal of*
621         *Odonatology*, *24*, 1–36.
622    QGIS Development Team. (2017). QGIS Geographic Information System. Open Source
623         Geospatial Foundation Project. http://qgis.org.
624    R Core Team. (2013). *R: A language and environment for statistical computing*. R
625         Foundation for Statistical Computing, Vienna, Austria.
626         https://www.gbif.org/tool/81287/r-a-language-and-environment-for-statistical-
627         computing
628    Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., & Montiglio, P.-O.
629         (2010). Personality and the emergence of the pace-of-life syndrome concept at the
630         population level. *Philosophical Transactions of the Royal Society B: Biological*
631         *Sciences*, *365*(1560), 4051–4063. https://doi.org/10.1098/rstb.2010.0208
632    Reynolds, J., & Souty-Grosset, C. (2011). *Management of freshwater biodiversity: Crayfish*
633         *as bioindicators*. Cambridge University Press.
634    RStudio Team. (2015). *RStudio: Integrated Development for R. RStudio, Inc., Boston, MA*
635         *URL http://www.rstudio.com/.*

636 Shochat, E., Warren, P. S., Faeth, S. H., McIntyre, N. E., & Hope, D. (2006). From patterns
637         to emerging processes in mechanistic urban ecology. *Trends in Ecology & Evolution*,
638         *21*(4), 186–191. https://doi.org/10.1016/j.tree.2005.11.019

639 Sniegula, S., Golab, M. J., Drobniak, S. M., & Johansson, F. (2018). The genetic variance but
640         not the genetic covariance of life-history traits changes towards the north in a time-
641         constrained insect. *Journal of Evolutionary Biology*, *31*(6), 853–865.
642         https://doi.org/10.1111/jeb.13269

643 Sniegula, S., Janssens, L., & Stoks, R. (2017). Integrating multiple stressors across life stages
644         and latitudes: Combined and delayed effects of an egg heat wave and larval pesticide
645         exposure in a damselfly. *Aquatic Toxicology*, *186*, 113–122.
646         https://doi.org/10.1016/j.aquatox.2017.02.029

647 Sniegula, S., Raczyński, M., Golab, M. J., & Johansson, F. (2020). Effects of predator cues
648         carry over from egg and larval stage to adult life-history traits in a damselfly.
649         *Freshwater Science*, *39*(4), 804–811. https://doi.org/10.1086/711374

650 Sokolovska, N., Rowe, L., & Johansson, F. (2000). Fitness and body size in mature odonates.
651         *Ecological Entomology*, *25*(2), 239–248. https://doi.org/10.1046/j.1365-
652         2311.2000.00251.x

653 Stoks, R., Swillen, I., & De Block, M. (2012). Behaviour and physiology shape the growth
654         accelerations associated with predation risk, high temperatures and southern latitudes
655         in Ischnura damselfly larvae. *Journal of Animal Ecology*, *81*(5), 1034–1040.
656         https://doi.org/10.1111/j.1365-2656.2012.01987.x

657 Tam, B. Y., Gough, W. A., & Mohsin, T. (2015). The impact of urbanization and the urban
658         heat island effect on day to day temperature variation. *Urban Climate*, *12*, 1–10.
659         https://doi.org/10.1016/j.uclim.2014.12.004

660 The General Directorate for Environmental Protection. (2018). *Orconectes limosus*. [accessed
661         17.02.2023] https://projekty.gdos.gov.pl/igo-orconectes-limosus; In Appendix A,
662         Smietana P, Bonk M, Solarz W [accessed 17.02.2023]
663         https://projekty.gdos.gov.pl/files/artykuly/126882/Orconectes-limosus_rak-
664         pregowany_EN_icon.pdf

665 Tüzün, N., & Stoks, R. (2021). Lower bioenergetic costs but similar immune responsiveness
666         under a heat wave in urban compared to rural damselflies. *Evolutionary Applications*,
667         *14*(1), 24–35. https://doi.org/10.1111/eva.13041

668 Van Dievel, M., Janssens, L., & Stoks, R. (2016). Short- and long-term behavioural,
669         physiological and stoichiometric responses to predation risk indicate chronic stress
670         and compensatory mechanisms. *Oecologia*, *181*(2), 347–357.
671         https://doi.org/10.1007/s00442-015-3440-1

672 Verheyen, J., Tüzün, N., & Stoks, R. (2019). Using natural laboratories to study evolution to
673         global warming: Contrasting altitudinal, latitudinal, and urbanization gradients.
674         *Current Opinion in Insect Science*, *35*, 10–19.
675         https://doi.org/10.1016/j.cois.2019.06.001

676 Warkentin, K. M. (2011). Plasticity of hatching in amphibians: Evolution, trade-offs, cues
677         and mechanisms. *Integrative and Comparative Biology*, *51*(1), 111–127.

678

679

**Competing interests**

The authors declare no conflicts of interest.

**Author contributions**

GP, RS and SS conceived and designed the experiments. GP and SS performed the experiments. GW analysed the data. GW and SS led the writing of the manuscript; all authors contributed critically to the drafts and gave final approval for publication.

**Date availability statement**

All data generated or analysed during this study are included in this article (and its supplementary information files).

700  **Figures.**

701  **Fig. 1.** Summary of the experimental design with a map of our sampled populations in

702  southern Sweden (high latitude) and southern Poland (central latitude). Geographic

703  distribution of *Ischnura elegans* in central and northern Europe is shown in grey and

704  occurrence of the spiny-cheek crayfish *Orconectes limosus* is depicted by red crosses. On the

705  left side, we show the design of the experiment 1 focusing on rural and urban populations

706  from central and high latitude reared at current (20 °C) and warming (24 °C) temperature.

707  Illustrative plot shows the significant interaction latitude $\times$ urbanization for the final larval

708  instar (F-0) mass. On the right side, we show the design of the experiment 2 focusing on

709  urban and rural populations from high latitude reared at current (20 °C), warming (24 °C),

710  and heat wave (28 °C) temperature and in a control or a predator cue treatment. Illustrative

711  plot shows the significant interaction temperature $\times$ predator cue for the growth rate until F-0.

712

713  **Fig 2**. Larval (A) mass $_{F0}$ and (B) growth rate $_{F0}$ (GR $_{F0}$) across urban and rural populations

714  and current (20 °C) and warming (24 °C) temperature. Larval (C) mass $_{F0}$ and (D) GR $_{F0}$

715  across urban and rural populations for females and males in experiment 1.

716

717  **Fig 3**. Principal component analysis showing evolutionary changes before the treatment with

718  predator cue in response to urbanization at current (20 °C) and warming (24 °C) for (A)

719  central- and (B) high latitude populations and plastic changes in response to temperature in

720  rural and urban populations for (C) central- and (D) high latitude populations (sexes pooled).

721  Rural and urban individuals are depicted by open circles and triangles respectively;

722  temperature by colours (blue = 20 °C and green = 24 °C); filled circles and triangles

723  correspond to the centroid of each group; solid lines connecting filled symbols represent the

724  vector.

725

726  **Fig 4**. Phenotypic plasticity trajectory in response to (A) urbanization at current (20 °C),

727  warming temperature (24 °C) and heat wave (28 °C) and to (B) temperature for rural and

728  urban populations. Rural and urban individuals are depicted by open circles and triangles

729  respectively; temperature by colours (blue = 20 °C, green = 24 °C and red = 28 °C); filled

730  circles and triangles correspond to the centroid of each group; solid lines connecting filled

731  symbols represent the vector.

27

**Table 1.** Results of the GLMM of the Experiment 1. Table shows effects of sex (females and males), latitude (central and high latitude), urbanization (rural and urban), temperature (20 °C and 24 °C) and of their interactions on larval development time, mass and growth rate (A) at entrance to F-0 (DT $_{F0}$, Mass $_{F0}$ and GR $_{F0}$) and (B) during the five-days exposure period to predator cues (GR $_{final}$). For each variable, we limited the analysis to the relevant predictors and interactions determined by the model selection analysis. The duration of the temperature treatment (post-winter 'thermal exposure duration') was included as covariate. Effects of random factors are not shown.
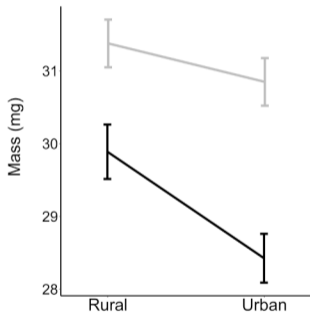
| | | At entrance to F-0 | | | | | Predator treatment |
|---|---|---|---|---|---|---|---|
| **A.** | | Dev. time $_{F0}$ | Mass $_{F0}$ | GR $_{F0}$ | **B.** | | GR $_{final}$ |
| Variables | df | $p$ | $p$ | $p$ | Variables | df | $p$ |
| Thermal exposure duration | 1 | / | **0.001** **(10.1) \*\*** | **0.001** **(1150) \*\*\*** | Thermal exposure duration | 1 | **0.001** **(10.6) \*\*** |
| Sex | 1 | / | **< 0.001** **(80.7) \*\*\*** | **0.001** **(75.2) \*\*\*** | Latitude | 1 | **0.022** **(5.25) \*** |
| Latitude | 1 | **< 0.001** **(14.2) \*\*\*** | **< 0.001** **(53.1) \*\*\*** | **0.001** **(112) \*\*\*** | Predator | 1 | **0.004** **(8.39) \*\*** |
| Temperature | 1 | **< 0.001** **(50.0) \*\*\*** | **< 0.001** **(76.7) \*\*\*** | **0.001** **(62.0) \*\*\*** | | | |
| Urbanization | 1 | / | 0.308 (1.03) | 0.236 (1.41) | | | |
| Latitude × sex | 1 | / | **0.036** **(4.40)** | 0.116 (2.47) | | | |
| Latitude × temperature | 1 | / | 0.050 (3.83) | **0.017** **(5.71) \*** | | | |
| Latitude × urbanization | 1 | / | **0.004** **(8.15) \*\*** | / | | | |
| Temperature × sex | 1 | / | 0.165 | / | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | (1.93) | | | | |
| Temperature × urbanization | 1 | / | **0.010** **(6.70) \*\*** | **0.047** **(3.93) \*** | | | |
| Urbanization × sex | 1 | / | **0.027** **(4.87) \*** | **0.032** **(4.59) \*** | | | |

Table shows p-values and Wald Chi-squared statistic (in parentheses) for each variable. Significance is indicated by \*\*\* $p < 0.001$, \*\* $p < 0.01$, \* $p < 0.05$.

**Table 2.** Results of the GLMM of the Experiment 2. Table shows effects of sex (females and males), urbanization (rural and urban), temperature (20 °C, 24 °C and 28 °C) and of their interactions on larval development time, mass and growth rate (A) at entrance to F-0 (Dev. time $_{F0}$, Mass $_{F0}$ and GR $_{F0}$) and (B) during the five-days exposure period to predator cues (GR $_{final}$). For each variable, we limited the analysis to the relevant predictors and interactions determined by the model selection analysis. The duration of the temperature treatment (post-winter 'thermal exposure duration') was included as covariate. Effects of random factors are not shown.

| | | At entrance to F-0 | | | | | Predator treatment |
|---|---|---|---|---|---|---|---|
| **A.** | | Dev. time $_{F0}$ | Mass $_{F0}$ | GR $_{F0}$ | **B.** | | GR $_{final}$ |
| Variables | df | $p$ | $p$ | $p$ | Variables | df | $p$ |
| Thermal exposure duration | 1 | / | **< 0.001** **(34.3) ***** | **< 0.001** **(1168) ***** | Thermal exposure duration | 1 | **< 0.001** **(12.5) ***** |
| Sex | 1 | **0.029** **(4.78) *** | **< 0.001** **(91.1) ***** | **< 0.001** **(84.8) ***** | Temperature | 2 | 0.122 (4.21) |
| Temperature | 2 | **< 0.001** **(43.0) ***** | **< 0.001** **(324) ***** | **< 0.001** **(316) ***** | Predator | 1 | 0.344 (0.89) |
| Urbanization | 1 | 0.063 (3.43) | / | / | Temperature × predator | 2 | **0.001** **(13.0) **** |
| Temperature × sex | 2 | / | **0.020** **(7.83) *** | **0.026** **(7.29) *** | | | |
| Temperature × urbanization | 2 | 0.31 (2.36) | / | / | | | |

Table shows p-values and Wald Chi-squared statistic (in parentheses) for each variable. Significance is indicated by *** p < 0.001, ** p < 0.01, * p < 0.05.
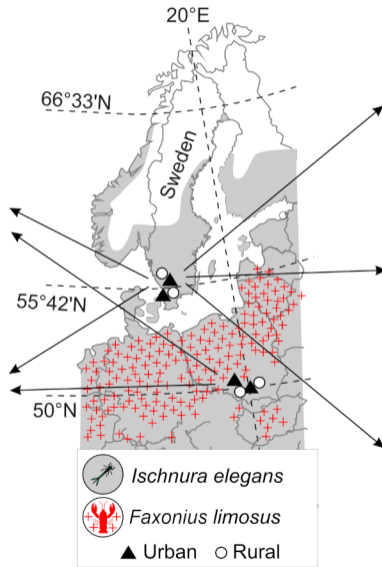
**Experiment 1**

Post-wintering

Effect of latitude and urbanization on mass at F-0
F-0

Mass (mg)

Region
Central latitude
High latitude

Distributions,
sampling

20°E

66°33'N
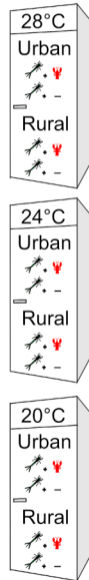
Sweden

55°42'N

50°N

*Ischnura elegans*

*Faxonius limosus*

▲ Urban    ○ Rural

24°C
Urban

Rural

20°C
Urban

Rural

28°C
Urban

Rural
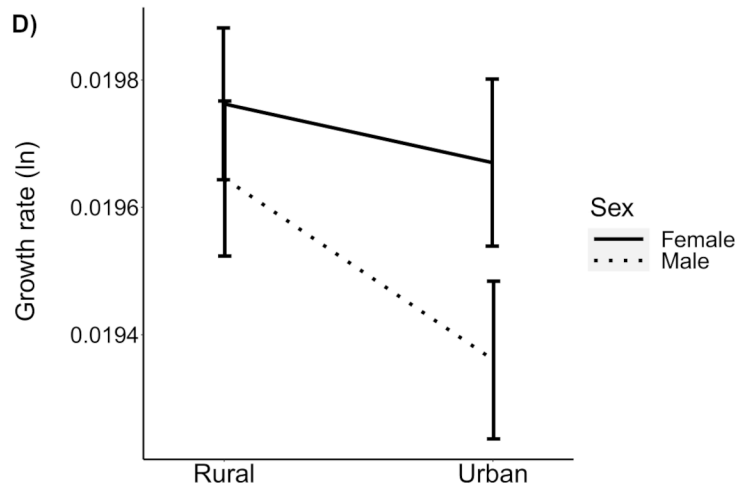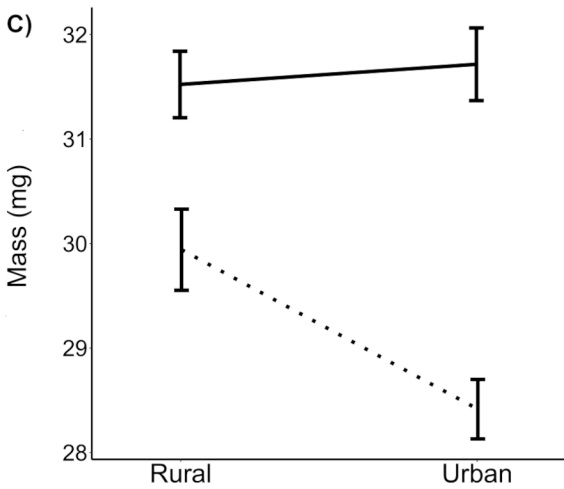
24°C
Urban

Rural

20°C
Urban

Rural

**Experiment 2**

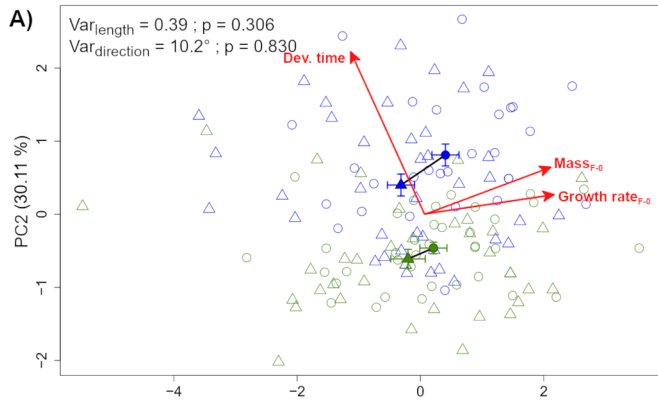Post-wintering

Effect of temperature and predator cue on
growth rate at F-0

Growth rate (ln)

Treatment
Control
Predator

**Central latitude**

**A)**
Var_length = 0.39 ; p = 0.306
Var_direction = 10.2° ; p = 0.830

Dev. time
Mass_{F-0}
Growth rate_{F-0}

PC2 (30.11 %)

**High latitude**

**B)**
Var_length = 0.70 ; p = 0.007
Var_direction = 132.4° ; p = 0.001

Dev. time
Mass_{F-0}
Growth rate_{F-0}

PC2 (32.75 %)

**C)**
Var_length = 0.27 ; p = 0.300
Var_direction = 15.1° ; p = 0.537

PC2 (30.11 %)

PC1 (68.81 %)

**D)**
Var_length = 0.26 ; p = 0.296
Var_direction = 39.9° ; p = 0.009

PC2 (32.75 %)

PC1 (66.69 %)

○ Rural 20 °C  ○ Rural 24 °C
△ Urban 20 °C  △ Urban 24 °C

A) Var_length = 0.02 ; p = 0.218
Var_direction = 3547.0 ; p = 0.001

B) Var_length = 0.720 ; p = 0.016
Var_direction = 10.0 ; p = 0.222

Growth rate$_{F-0}$
Mass$_{F-0}$
Dev. time

PC2 (33.32 %)
PC1 (66.07 %)
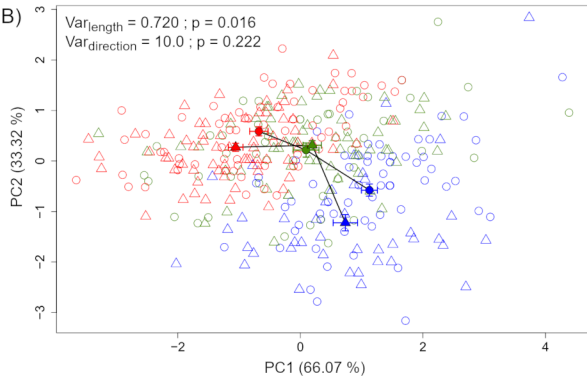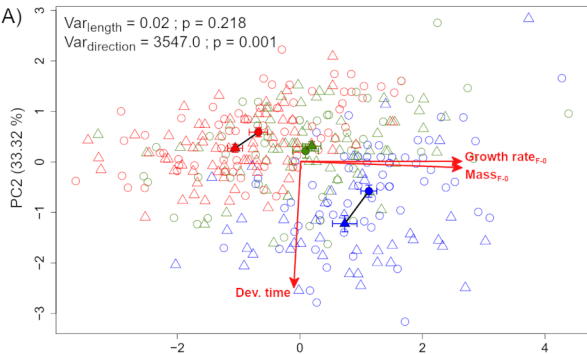
○ Rural 20 °C   ○ Rural 24 °C   ○ Rural 28 °C
△ Urban 20 °C   △ Urban 24 °C   △ Urban 28 °C