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Genetic differentiation in pesticide resistance between urban and rural populations of a nontarget freshwater keystone interactor, *Daphnia magna*

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Abstract

There is growing evidence that urbanization drives adaptive evolution in response to thermal gradients. One such example is documented in the water flea *Daphnia magna*. However, organisms residing in urban lentic ecosystems are increasingly exposed to chemical pollutants such as pesticides through run-off and aerial transportation. The extent to which urbanization drives the evolution of pesticide resistance in aquatic organisms and whether this is impacted by warming and thermal adaptation remains limitedly studied. We performed a common garden rearing experiment using multiple clonal lineages originating from five replicated urban and rural *D. magna* populations, in which we implemented an acute toxicity test exposing neonates (<24h) to either a solvent control or the organophosphate pesticide chlorpyrifos. Pesticide exposures were performed at two temperatures (20°C vs. 24°C) to test for temperature-associated differences in urbanization-driven evolved pesticide resistance. We identified a strong overall effect of pesticide exposure on *Daphnia* survival probability (−72.8 percentage points). However, urban *Daphnia* genotypes showed higher survival probabilities compared to rural ones in the presence of chlorpyrifos (+29.7 percentage points). Our experiment did not reveal strong temperature x pesticide or temperature x pesticide x urbanization background effects on survival probability. The here observed evolution of resistance to an organophosphate pesticide is a first indication *Daphnia* likely also adapts to pesticide pollution in urban areas. Increased pesticide resistance could facilitate their population persistence in urban ponds, and feed back to ecosystem functions, such as top-down control of algae. In addition, adaptive evolution of nontarget organisms to pest control strategies and occupational pesticide use may modulate how pesticide applications affect genetic and species diversity in urban areas.

KEYWORDS
chlorpyrifos, *Daphnia magna*, organophosphate pesticides, pesticide resistance, urban evolution

1 | INTRODUCTION

Humans ubiquitously impact natural ecosystems in various ways (Hendry et al., 2017; Palumbi, 2001). Strong forms of human-driven land conversion and habitat and microclimate alterations are concerted in cities (Alberti et al., 2020; Grimm et al., 2008; Parris, 2016); urbanization modifies the physical, chemical, and biological characteristics of natural habitats, structurally and functionally changes connectivity patterns among biological communities, induces disturbances of which many are novel (e.g. chemical contaminants and noise), and finally alters biotic interactions as a consequence of changes in native species composition and the introduction of non-native species. The impact of urbanization on species distributions and diversity, community assembly, and interspecific trait variation has been extensively monitored (Aronson et al., 2014, 2016; Dallimer et al., 2012; Merckx et al., 2018; Piano et al., 2017). In recent years, it has become clear that cities moreover drive contemporary evolution (Donihue & Lambert, 2015; Johnson & Munshi-South, 2017). A large number of observations of urban evolution focuses on neutral evolution stemming from the impact of habitat fragmentation, among-city connectivity hubs, and population size changes on gene flow and genetic drift (Miles et al., 2019; Munshi-South & Richardson, 2020). In addition, evidence on adaptive evolution in response to urban temperature gradients (e.g. acorn ants: Diamond et al., 2017; Martin et al., 2019; water fleas: Brans et al., 2018; Brans, Jansen, et al., 2017), fragmentation (e.g. holy hawksbeard: Cheptou et al., 2017), and specific chemical pollution (e.g. polychlorinated biphenyl resistance in tomcod: Wirgin et al., 2011; and killifish: Oziolor & Matson, 2018; Whitehead et al., 2012) is growing incessantly.

One example of urban adaptation is documented in the water flea *Daphnia magna* (Brans & De Meester, 2018; Brans, Engelen, et al., 2018; Brans, Govaert, et al., 2017; Brans, Jansen, et al., 2017). *Daphnia magna*, a large-bodied cladoceran, resides in lakes and ponds, and is an important interactor in the freshwater aquatic food web; *Daphnia* exerts top-down control on algae and can prevent the occurrence of nuisance algal and bacterial blooms as a consequence of its size-related grazing efficiency (Chislock et al., 2013; Gianuca et al., 2016). Compared to rural populations, urban populations of water fleas show an evolved higher heat tolerance (Brans, Jansen, et al., 2017), faster pace-of-life (Brans & De Meester, 2018), altered stress physiology and a higher energy budget (Brans, Engelen, et al., 2018). Urban ponds in the study region (Flanders, Belgium) are significantly warmer compared to rural ponds (differences in summer mean and maximum temperature range between 3°C–4°C; Brans, 2018), driving observed genetic increases in heat tolerance, haemoglobin concentrations, as well as body size reductions (Brans, Jansen, et al., 2017). While evidence on thermal adaptation in urban water fleas is profound, to what extent they adapt to pollution in urban areas, and whether interactions between warming and pollution have effects on biotic evolutionary and ecological responses (Cuenca Cambronero et al., 2018; Moe et al., 2013; Noyes et al., 2009; Theys et al., 2020) is not known in this study system.

Residing in closed ecosystems such as ponds and pools, aquatic biota are exposed to a multitude of stressors, including chemical contaminants such as pesticides (Allinson et al., 2015; Meftaul et al., 2020). In cities, pesticides enter freshwater ecosystems via run-off and aerial transportation (Allinson et al., 2015; Meftaul et al., 2020; Wittmer et al., 2010), after application in public places, such as parks and schools, or for occupational purposes (e.g. domestic indoor use, private gardens) (Meftaul et al., 2020; Pang et al., 2002). After prohibition of organochlorines (e.g. DTT) in the 1970s, pyrethroids and organophosphate pesticides (e.g. chlorpyrifos, glyphosate, malathion, diazinon and carbaryl) were extensively and commonly used to suppress nuisance pests (insects, fungi) and unwanted weeds in municipal, business and domestic environments (Heudorf et al., 2004; Julien et al., 2008; McKelvey et al., 2013; Vijftigschildt et al., 2005; Wittmer et al., 2010). Such pesticide exposures in combination with warming (as a consequence of urban heat islands) in cities could synergistically alter evolutionary and ecological responses in *Daphnia*. For example, warming and heat stress could increase the toxicity of a pesticide, directly or via the increased uptake of the toxicant as a consequence of altered metabolic processes and, vice versa, toxicant exposure could limit acclimation or adaptation to warming and heat stress (Holmstrup et al., 2010; Hooper et al., 2013; Moe et al., 2013; Noyes et al., 2009; van den Brink et al., 2018).

Daphnia is a well-established ecotoxicological model system in ecological risk assessment of pesticides by means of standardized acute and chronic toxicity testing (Brock & Van Wijngaarden, 2012; Huang et al., 2020; Palma et al., 2008). Organophosphate pesticides impact *Daphnia* survival, embryological development, fertility, endocrine function, life history changes, and stress physiological parameters such as enzymatic defence and energy budget (Ferrario et al., 2018; Jeon et al., 2013; Palma et al., 2009; Pérez et al., 2015; Silva et al., 2018; Song et al., 2017; Wang et al., 2009; Zalizniak & Nugegoda, 2006). Impacts tested in more realistic settings often focus on the assessment of relative sensitivities of multiple organism groups (Cuppen et al., 2002; Echeverri-Jaramillo et al., 2020; Gaizick et al., 2001; Pérez et al., 2015), the impact of individual interacting environmental variables on model organisms (e.g. food concentration, shelter availability; Augusiak & van den Brink, 2016), the toxicity of pesticide mixtures, drainage ditch water or rain water samples on nontarget species (Cáceres et al., 2007; Hasenbein et al., 2017; Sakai, 2002; Wood & Stark, 2002) or ecosystem level interactions in standardized indoor or outdoor mesocosms (Kersting & van Wijngaarden, 1992; Knillmann et al., 2012; López-Mancisidor et al., 2008; van den Brink et al., 1995; Zafar et al., 2011). While such experiments are crucial to improve ecological risk assessment of pesticides and the mechanistic understanding of their specific modes of action and detrimental effects, they often overlook the possibility that populations can evolve resistance to applied toxicants (e.g. using 'naïve' pooled populations and communities of zooplankton, Rumschlag et al., 2020). Evolved pesticide resistance in natural populations of *Daphnia* in response to agricultural pesticide application was shown both across spatial gradients (organophosphates: Bendis & Relyea, 2014; carbamates: Coors et al., 2009; Jansen et al., 2015)

as well as in response to historical contamination (resurrection ecology, organophosphates: Simpson et al., 2017). Such adaptive evolutionary responses can have ecosystem-wide buffering effects (e.g. shown in *D. pulex*: Bendis & Relyea, 2016a, 2016b) and could potentially occur in cities, both in response to pesticide pollution, warming, and a combination of both.

Studies on evolved pesticide resistance in urban areas are so far focused on disease vectors and pests (Zhu et al., 2016) such as malaria and dengue transmitting mosquitos (Kamdem et al., 2017; Li et al., 2018; Macoris et al., 2003; Pereira Lima et al., 2003), bed bugs (Romero & Anderson, 2016), and cockroaches (Wu & Appel, 2017). Nevertheless, nontarget organisms, especially those residing in closed aquatic systems, such as ponds and pools, could also evolve pesticide resistance. For example, exposure to contaminated water way run-off leads to pyrethroid resistance in freshwater crustaceans (amphipods; Major et al., 2018). So far, only one specific example is available on the evolution of resistance to pesticides in nontarget species in a multi-stressor urban context (warming x pesticide exposure) (Tüzün et al., 2015); using a common garden approach, urban and rural damselfly nymphs (*Coenagrion puella*) were exposed to sublethal concentrations of chlorpyrifos at two different temperatures (20°C vs. 24°C—mimicking observed urban heat islands in the study region, Flanders, air: Wouters et al., 2017, water: Brans, Engelen, et al., 2018). Upon exposure to chlorpyrifos, urban genotypes increase their activity and keep food intake constant at both 20°C and 24°C, in contrast to rural genotypes, which show a reduction in both behavioural parameters at both temperatures. The development of such pesticide resistance in nontarget organisms across multiple trophic levels could modulate genetic and species diversity, as well as ecosystem functions and services in urban areas (Bendis & Relyea, 2016b; Des Roches et al., 2020).

This study primarily aims to explore whether urban *D. magna* evolved resistance to pesticides and to what extent responses are magnified by warming (Knillmann et al., 2013; Moe et al., 2013; Theys et al., 2020) and/or shaped by thermal adaptation (Op de Beeck et al., 2017; Dinh Van et al., 2013). We performed a common garden rearing experiment using replicated urban and rural populations of *Daphnia magna*. We assessed survival after an acute exposure (48h) to the model organophosphate pesticide chlorpyrifos, at two different temperatures, 20°C and 24°C (mimicking the urban heat island effect observed in ponds in the study region, Brans, Engelen, et al., 2018). Chlorpyrifos is a broad-spectrum organophosphate insecticide that inhibits acetylcholinesterase (Giddings et al., 2014) and has been applied in urban areas worldwide (Heudorf et al., 2004; McKelvey et al., 2013; Vijftigschildt et al., 2005). It is considered to be a priority pollutant in the European Water Framework as per Directive 2000/60/CE. Chlorpyrifos has been shown to induce signals of adaptive phenotypic trait change in urban damselflies in the same study region (Flanders, Belgium) and was not yet prohibited during the time period of sampling populations of *D. magna* and *Coenagrion puella* (2013–2015; Speedy sampling, see also Merckx et al., 2018). We hypothesize that (1) urban genotypes will have a higher survival when exposed to chlorpyrifos as a consequence of

evolved resistance to organophosphates, (2) survival in response to chlorpyrifos might be temperature-dependent due to a higher toxicity of chlorpyrifos at higher temperatures, and (3) temperature-dependent resistance responses differ between urban and rural genotype sets as a consequence of thermal adaptation in urban populations.

2 | MATERIAL AND METHODS

2.1 | Study populations, experimental design and pre-experimental conditions

Daphnia magna populations originated from five urban and five rural populations (Table S1) located in Flanders (Belgium) and are a subset of the 13 populations used in Brans, Jansen, et al. (2017) for which clonal lineages have been established in the laboratory (2012–2013, Speedy sampling campaign (Brans, Govaert, et al., 2017; Brans, Jansen, et al., 2017; Merckx et al., 2018). Flanders is a densely population region (ca. 371, amounting to 693 inhabitants/km², IBZ, 2021). Urbanization levels were assessed based on the percentage built-up area (BA) in the regional surroundings of the pond (3200 m radius as in Brans & De Meester, 2018; Brans, Govaert, et al., 2017; Brans, Jansen, et al., 2017; Brans et al., 2018), based on the Large-scale Reference Database (LRD, 2013). Urban populations are characterized by >10% BA and nonurban ('rural') populations <5% (Table S1; range urban: 14.12%–40.44% BA; range rural: 0.63%–4.38%; see also Figure 1 in Brans & De Meester, 2018). Percentage BA does not include roads and parking lots, which results in ponds characterized by more than 10%–15% of BA or higher (in this case all urban ponds >14% BA) being already substantially urbanized (Brans & De Meester, 2018). Percentage BA has shown to be a reliable proxy of urbanization as it, in the specific study region, positively correlates with impervious substrate cover like roads and artificial constructions such as viaducts bridges, and is negatively associated with the amount (area) of semi-natural habitat (Piano et al., 2020). In addition, rural nonurban locations had to meet the criterion of minimum 20% biologically valuable area (based on the Biological Value Map Flanders that includes land cover and vegetation mapping according to a biological valuation based on ecological criteria such as biodiversity, rarity, vulnerability and replaceability of the biotope; Brans, Govaert, et al., 2017; Saeger et al., 2017) in their regional surroundings (3200 m radius) to prevent sampling populations in nonurban agricultural areas.

Individuals from the five clonal lines from each population (*n* = 50) were inoculated in 200-mL glass jars filled with dechlorinated tap water, reared under standardized laboratory conditions (20°C, 16:8 L:D photoperiod) and fed *ad libitum* with the unicellular green algae *Acutodesmus obliquus* (1 × 10⁵ cells/mL, coinciding with the Incipient Limiting Level of 1 mg C/L). 80% of the medium was refreshed every other day. After the first generation, lineages were split into eight independent culturing units, using parthenogenetic offspring of mother individuals, in order to generate replicated

lines that could later be exposed to the pesticide treatment (control/chlorpyrifos) and temperature treatment (20°C/24°C) in a full factorial crossing. Each clone × temperature × pesticide treatment combination was replicated twice. To obviate interference from (grand)maternal effects, these eight clonal replicates were cultured for a minimum of two consecutive generations before pesticide exposures took place. Newborn juveniles were eliminated to prevent overcrowding. Second to fourth clutch juveniles were used to start up new generations or to start up the experimental exposure phase (see further). This overall design resulted in 2 urbanization classes (rural/urban) × 5 populations × 5 clones × 2 pesticide treatments (control/pesticide) × 2 temperatures (20°C/24°C) × 2 replicates = 400 experimental units. Due to the continuous infestation of one clone (u2.5) with a parasitic infection and consequently weak offspring production, and eventually mortality, it was completely excluded from the experiment (resulting in a total number of 392 experimental units).

2.2 | Pesticide exposure and survival

We exposed five juveniles (<24h old but older than 5h) per experimental unit according to OECD guidelines (2004) to either a control (solvent) or chlorpyrifos (CAS: 2921-88-2, purity >99%, Sigma-Aldrich; nominal concentration of 0.67 µg/L), at both 20°C and 24°C, using 100-mL glass recipients filled with 50 mL of the exposure medium (control medium/pesticide medium—no feeding, OECD, 2004). The pesticide exposure medium was prepared daily from a stock solution (1 mg/mL, Sigma-Aldrich Chlorpyrifos powder >99% purity dissolved in absolute ethanol (100%), stored in an dark brown glass bottle and kept at 4°C in dark conditions). The stock solution was diluted first with Mili-Q (step 1, intermediate stock) and secondly with dechlorinated tap water (step 2, exposure medium) to achieve the nominal concentration of 0.67 µg/L in the exposure medium. For the solvent control medium the same amount of absolute ethanol (100%) was used in the first dilution step (instead of the pesticide). The nominal 0.67 µg/L chlorpyrifos concentration was set after an initial range finding pilot exposure with nominal chlorpyrifos concentrations ranging between 0.3 and 0.85 µg/L, using a pooled batch of animals (urban and rural mixed). The chosen concentration lies between the range for which EC50 values of *D. magna* have been found (0.6 µg/L in Moore et al., 1998; 0.25 µg/L in Naddy et al., 2009; 0.74 µg/L in Palma et al., 2008; 0.49 µg/L in Raymundo et al., 2019) and within the observed range of concentrations found in European surface waters in the wider region (waters: 95% confidence interval = [0.07, 0.69 µg/L]) (Schulz, 2004; Stehle & Schulz, 2015). Immobilization was assessed after 24 and 48h (OECD, 2004), with the criterion of minimally antennae movement to be clearly observed. Upon counting immobilized animals, we noticed six instead of five juveniles to be present in four (out of 392) experimental units. Given we follow OECD acute toxicity assay guidelines, which require pesticide exposures to be performed in absence of food, we confirmed the potential impact (e.g. differences in energy acquisition)

of this manual error to be minimal (see further). In addition, newborn juveniles (<24h old) perform limited to no grazing activity (Cowgill et al., 1984).

2.3 | Statistical analyses

We use a Bayesian binomial generalized linear mixed model to estimate the effect of pesticide treatment (control/pesticide), urbanization background (rural/urban) and temperature (20°C/24°C), as well as their interactions, on *D. magna* survival probability after 48h. More specifically, we assume that the number of surviving individuals y_i of the i'th replicate follows a binomial distribution:

$$y_i \sim \text{Binomial}\left(N_i, p_i\right),$$

where N_i is the replicate's initial number of individuals (5, or exceptionally 6), and p_i is the individual survival probability, modelled through the following logit link function:

$$\log\left(\frac{p_i}{1-p_i}\right) = \beta_0 + CPF_i \bullet \beta_{CPF} + URB_i \bullet \beta_{URB} + T_i \bullet \beta_T$$
$$+ CPF_i \bullet URB_i \bullet \beta_{CPF:URB} + CPF_i \bullet T_i \bullet \beta_{CPF:T} + URB_i \bullet T_i \bullet \beta_{URB:T}$$
$$+ CPF_i \bullet URB_i \bullet T_i \bullet \beta_{CPF:URB:T} + CPF_i \bullet b_{pop(i)} + CPF_i \bullet b_{clone(i)},$$

where β_0 is the intercept, CPF_i indicates whether the individuals of the i'th replicate were exposed to chlorpyrifos (0.5) or not (−0.5), URB_i indicates whether the individuals of the i'th originated from a rural (−0.5) or urban (0.5) population, T_i indicates whether the individuals of the i'th replicate were exposed to 20°C (−0.5) or 24°C (0.5), the $\beta_{\bullet}$-parameters represent the corresponding main and interaction effects, and $b_{pop(i)}$ and $b_{clone(i)}$ are the population- and clone-level random effects corresponding to the i'th replicate. As the input variables CPF, URB and T are centred around zero, the intercept β_0 represents the average condition.

Heterogeneity among populations with respect to sensitivity to chlorpyrifos exposure is modelled through a normally distributed population-level random effect b_{pop}:

$$b_{pop} \sim \text{Normal}\left(0, \sigma_{pop}\right),$$

where σ_{pop} is the population-level standard deviation. Remaining heterogeneity among clones that is not explained by the population-level random effects is modelled by means of a clone-level random effect b_{clone}. As a preliminary exploration revealed some presumably outlying clone-specific responses, we model the clone-level random effects with a Student's t-distribution:

$$b_{clone} \sim \text{StudentT}\left(\nu, 0, \sigma_{clone}\right),$$

where ν is the number of degrees of freedom, and σ_{clone} is the clone-level scale parameter (which we call standard deviation below for simplicity). For low values of ν, the Student's t-distribution has substantially fatter tails compared to the normal distribution, offering robustness against unusual observations. As $\nu \to \infty$, the Student's

t-distribution approximates the normal distribution. By treating v as an unknown quantity (to be estimated as part of the model), the thickness of the distribution's tail is tuned according to the data and the prior (Gelman et al., 2014).

We choose weakly informative priors for all model parameters:

$$\beta \sim \text{StudentT}(3, 0, 5),$$

$$\sigma_{pop}, \sigma_{clone} \sim \text{StudentT}^+(3, 0, 5),$$

$$v \sim \text{Gamma}(2, 0.1).$$

As the priors on β (the vector of all regression coefficients), σ_{pop} and σ_{clone} place most prior mass around zero, they mainly have a regularizing effect on the posteriors distribution (i.e. shrinking estimates towards 0 unless the data indicates otherwise). The Gamma-prior on v is chosen following the recommendations by Juárez and Steel (2010).

We implemented this Bayesian robust generalized linear mixed model using the probabilistic programming language Stan and performed Markov chain Monte Carlo (MCMC) sampling through the rstan v.2.21.2 package (Stan Development Team, 2020) in R v.4.0.3 (R Core Team, 2020). Stan performs Bayesian inference by means of dynamic Hamiltonian Monte Carlo (HMC), a gradient-based MCMC sampler (Carpenter et al., 2017). We ran four chains with 10,000 iterations each, of which the first 5,000 were discarded as warmup. The resulting 20,000 posterior samples are summarized using posterior medians and 95% equal-tailed credible intervals (bounded by the 2.5% and 97.5% samples from the distribution), unless otherwise specified. We used the tidybayes v.2.3.1 package (Kay, 2020) to visualize the posterior distributions.

We assessed model convergence both visually by means of traceplots and numerically by means of effective sample sizes, divergent transitions and the Potential Scale Reduction Factor, for which all parameters had $\hat{R} < 1.01$ (Vehtari et al., 2019). We performed posterior predictive checks to assess goodness-of-fit and performed a sensitivity analysis to assess the influence of the chosen priors (see Supporting Information, Figures S1–S13).

The full code for the analyses is available through https://github.com/mfajgenblat/brglmm-chlorpyrifos.

As mentioned, there were four experimental units which contained six instead of five juveniles. Analyses of the data excluding these four observations did not qualitatively change the results (see further and Figure S14, Supplementary Information).

3 | RESULTS

In this experiment, *Daphnia magna* individuals faced important reductions in survival probability at 48h upon exposure to chlorpyrifos, with an estimated effect of –7.86 (95% CrI [–9.93, –6.37]) on the logit scale (Figure 1). On the probability scale, the marginal effect (at the mean values of the other variables) of chlorpyrifos exposure corresponds to a reduction of 72.80 percentage points (95% CrI [0.50, 0.88]) in survival probability: survival is 99.90% (95% CrI [99.42, 99.99]) without exposure and drops to 27.07% (95% CrI [11.66, 49.70]) with exposure (Figures 1, 2). By contrast, the main effects of urbanization and temperature on survival probability are estimated to be much weaker, respectively –0.35 (95% CrI [–3.16, 2.42]) and –1.01 (95% CrI [–3.03, 0.70]) (Figure 1). On the probability scale, an urban population origin and a 24°C temperature confers a marginal reduction in survival probability of, respectively 1.41 (95%
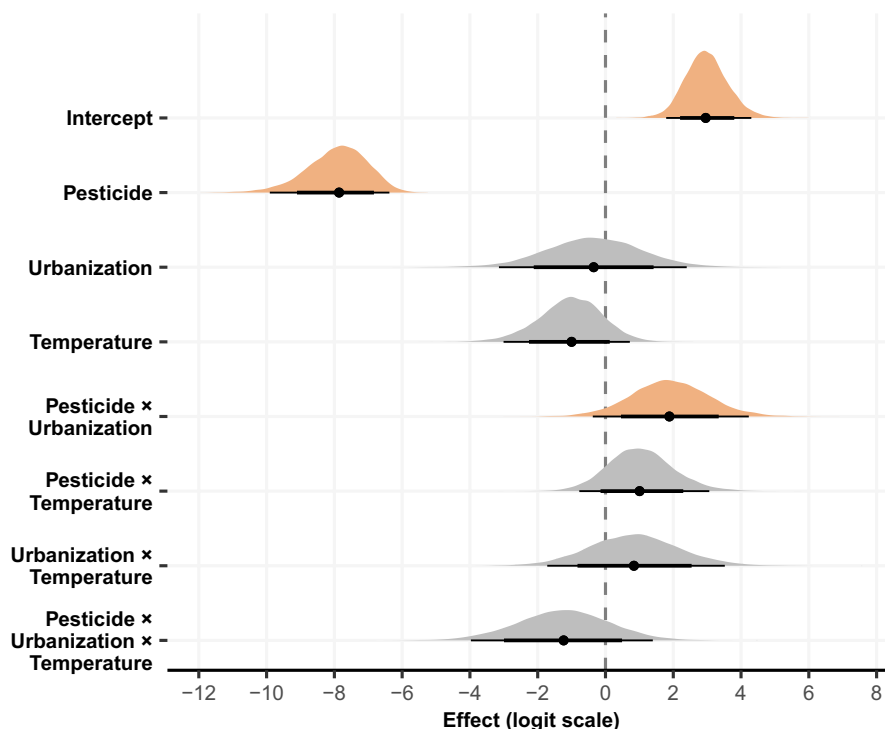
FIGURE 1 Estimated main and interaction effects on survival (logit scale). Bayesian posterior densities are represented by density plots, 80% and 95% credible intervals by horizontal lines, and posterior medians by black dots. Negative values correspond to a decreased survival probability, while positive values correspond to an increased survival probability, relative to the average condition, population and clone. Variables for which the posterior probability that their effect is either positive or negative exceeds 0.95 (given model and data) are shown in orange
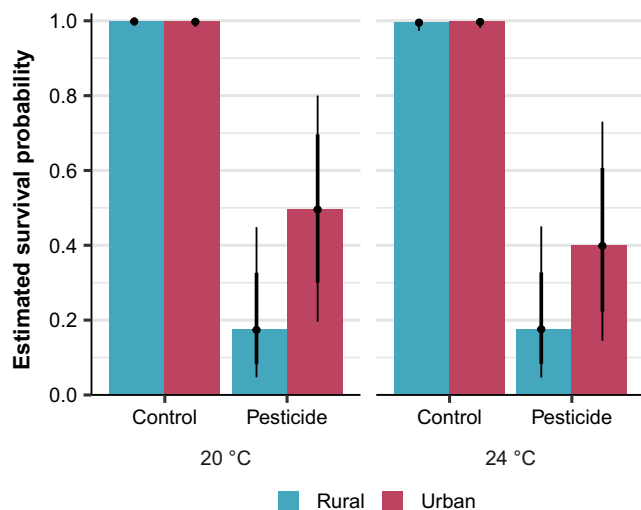
FIGURE 2 Additive visualization of Figure 1: estimated survival probability after 48h for each combination of urbanization level (rural, blue vs. urban, red), chlorpyrifos exposure treatment (control vs. pesticide), and temperature treatment (20°C vs. 24°C). 80% and 95% credible intervals are shown by vertical lines and posterior medians by black dots

CrI [−20.29, 18.02]) and 4.43 (95% CrI [−5.20, 15.30]) percentage points, at the mean values of the other variables.

However, we identified a positive interaction effect between chlorpyrifos exposure and urbanization, with 95.16% posterior probability (Figure 1). This effect is estimated to be 1.91 (95% CrI [−0.38, 4.22]) on the logit scale. Equivalently, urban *Daphnia* individuals exposed to chlorpyrifos have a 44.48% (95% CrI [16.71, 75.90]) marginal probability of survival, compared to 14.76% (95% CrI [3.78, 40.66]) for rural individuals, considering the mean values of the other variables (Figures 1, 2). The interactions effects between chlorpyrifos exposure and temperature and between urbanization and temperature are estimated to be smaller; 1.02 (95% CrI [−0.75, 3.06]) and 0.84 (95% CrI [−1.75, 3.52]) on the logit scale, respectively (Figure 1).

The three-way interaction between chlorpyrifos exposure, urbanization and temperature is estimated to be negative with 81.73% posterior probability and has an estimated effect of −1.24 (95% CrI [−4.01, 1.40]) on the logit scale (Figure 1). The estimated survival probabilities for each of the eight considered treatment combinations are graphically summarized in Figure 2.

Furthermore, we identified important heterogeneity with respect to chlorpyrifos sensitivity among populations and clones, which was expected as we included replicated urban and rural populations and clones. The estimated posterior medians for the population- and clone-level standard deviations equal 1.25 (95% CrI [0.59, 2.60]) and 1.12 (95% CrI [0.73, 1.58]), respectively. The estimated random effects for all clones, corrected for urbanization level (through the fixed effects), are visualized in Figure S15 (Supplementary Information) and show some particularly striking deviations compared to other clones in one rural and one urban population. These findings indicate that within populations, among-clonal differentiation in tolerance

responses to the pesticide can be found, sometimes to important extents, and thus reflect evolutionary potential. This is a common observation in *Daphnia* and in concordance with studies on thermal adaptation in response to urban heat islands and climate change (Brans, Jansen, et al., 2017; Geerts et al., 2015). The use of a robust Student's *t*-distribution random effects specification accommodates these outlying observations by preventing a strong influence on the location and scale parameter estimates. Indeed, the estimated number of degrees of freedom for the Student's *t*-distribution used for the clone-level random effects equals 14.76 (95% CrI [2.83, 53.64]), corresponding to a distribution with somewhat fatter tails than the normal distribution.

4 | DISCUSSION

In general, *Daphnia* survival was impaired by the acute exposure (48h) to the organophosphate pesticide chlorpyrifos compared to the control (−72.8 percentage points). However, we found strong evidence for a pesticide x urbanization effect, reflecting that urban water fleas showed a higher survival probability (+29.7 percentage points) compared to their rural counterparts in the presence of chlorpyrifos. While survival in response to the chlorpyrifos treatment was strongly impacted by the evolutionary urbanization background of the populations, we found no support for urbanization-driven survival differences at different exposure temperatures (20°C vs. 24°C). We here discuss the observed effects in more detail.

Daphnia survival probability after acute exposure (OECD, 2004) was strongly impacted by chlorpyrifos at the used nominal test concentration of 0.67 µg/L (−72.8 percentage points, Figure 1). This is in concordance with previous observations of the range of chlorpyrifos concentrations for which EC50 values in *Daphia* have been reported (Huang et al., 2020; Palma et al., 2008). Importantly, we found that urban *Daphnia* genotypes had a higher survival probability when exposed to chlorpyrifos compared to rural genotypes (Figures 1 and 2). As expected, survival in the control was high for both rural and urban genotype sets (95.8% and 94.2%, respectively), without support for any important difference. However, animals originating from rural ponds show an expected drop of 29.7 percentage points in survival probability in the presence of the organophosphate pesticide compared to urban animals (from 44.48% marginal survival probability in urban to 14.76% in rural). These results confirm the first hypothesis and indicate evolved pesticide resistance in urban water fleas. The present observation supports studies that show species in urban areas can evolve resistance to pesticide applications (Zhu et al., 2016), mostly confirmed for target species (Li et al., 2018; Macoris et al., 2003; Romero & Anderson, 2016). For example, mosquitos of the *Anopheles gambiae* complex (malaria vectors) in urban areas developed resistance to insecticides (Kamdem et al., 2017), which was supported by genome scans showing selective sweeps on genes whose functions include epidermal growth, olfaction, cuticle formation, and resistance and detoxification of insecticides (Kamdem et al., 2017). More importantly, our study exemplifies the

evolutionary impacts of pesticide applications on nontarget species in cities, which was so far limitedly demonstrated. The present results correspond strikingly to the urbanization-driven evolution of organophosphate resistance in damselfly nymphs sampled in the same region (Flanders, Belgium—2013–2015) and exposed to the same pesticide (Tüzün et al., 2015). In a common garden experiment with F0 nymphal stages, Tüzün et al. (2015) demonstrated that urban damselfly nymphs increase activity and maintain feeding behaviour under chronic exposure to chlorpyrifos (2 μg/L), compared to rural damselfly nymphs. While different in set-up (testing sublethal concentrations in a chronic exposure vs. acute toxicity testing, damselfly nymphs vs. water fleas, respectively), both studies build up the evidence for urbanization-driven evolution of organophosphate resistance in aquatic nontarget species in Flanders (Belgium). Importantly, concentrations applied in Tüzün et al. (2015) and our study highlight a drastic difference in species sensitivity to organophosphate pollution (specifically chlorpyrifos in both cases) across interacting species (damselfly nymphs are common predators of water fleas). A contamination of urban waters at concentrations nonlethal to damselfly nymphs would imply already drastic mortality in one of their important prey components, which could potentially have indirect effects on biotic responses to pesticides observed in urban damselflies. We therefore advocate the need to perform experiments on multiple (interacting) species across food webs, both in ecotoxicological studies and in studies that aim to disentangle and understand eco-evolutionary feedbacks in response to anthropogenic disturbances, such as urbanization (De Meester et al., 2019).

Though speculative, we present a number of potential mechanisms based on literature that could underlie the genetic differentiation in pesticide resistance between urban and rural *Daphnia magna* genotypes and that need further investigation in future research. Urban water fleas from the same urban study gradient evolved a higher energy budget, reflected in higher concentrations of protein, fat and carbohydrates in their body tissue (Brans, Stoks, et al., 2018). Higher net levels of energy compounds can enable an increased energy allocation to detoxification mechanisms under stress (De Coen & Janssen, 2003; Sokolova, 2013). Short-term exposure of *Daphnia* to pesticides and metals indeed induces significant depletions in energy compounds (De Coen & Janssen, 2003; Sancho et al., 2009), and thus, evolved higher constitute levels of such compounds could be an adaptive buffer mechanism for toxicant adaptation (Brans, Stoks, et al., 2018). Given their high evolutionary potential and shown adaptations to a variety of anthropogenic stressors (Coors et al., 2009; Geerts et al., 2015; Jansen et al., 2015; Messiaen et al., 2010), selection on genes involved in detoxification and cellular protection, as observed in mosquitos (Kamdem et al., 2017), could occur in urban water fleas, but needs verification via genomic approaches.

We found no strong evidence for temperature-mediated responses to the pesticide exposure such as warming-induced toxicant sensitivity (Moe et al., 2013; Noyes et al., 2009). First, *Daphnia* survival probability when exposed to chlorpyrifos did not differ between 20°C and 24°C (pesticide × temperature, Figure 1) and is thus not in line with our second hypothesis. Warming-induced increased

toxicant sensitivity has been reported for chlorpyrifos before, including exposures to increases in mean temperature and temperature variation (freshwater isopods: Theys et al., 2020; *Daphnia*: Barbosa et al., 2017; Cuenca Cambronero et al., 2018). While we applied a 4°C temperature increase, it is argued the combination of both mean temperatures combined with daily temperature variation will more strongly impact biotic responses to pesticides, as organisms experience more extreme temperatures and thus also are challenged at more extreme physiological limits (Verheyen et al., 2019). In addition, while urban water flea genotypes have a higher pesticide resistance compared to rural genotypes, the data do not strongly support an urbanization × pesticide × temperature response (Figure 1), similarly as reported for damselfly nymphs (Tüzün et al., 2015). Urban water fleas are exposed to higher mean temperatures in urban ponds (Brans, Engelen, et al., 2018) and show signals of urban thermal adaptation (increased heat tolerance and haemoglobin concentrations, smaller body size) (Brans, Jansen, et al., 2017). We expected that chlorpyrifos resistance in urban *Daphnia* would be higher at 24°C compared to resistance in rural animals at that temperature, given thermal adaptation can offset pesticide toxicity under warming conditions (Op de Beeck et al., 2017; Dinh Van et al., 2013). Possibly, such responses would be more strongly elicited under more stressful thermal conditions such as heat spikes or heatwaves (Tüzün & Stoks, 2020). We did not apply a heat spike or heatwave in our experimental design as the exposure would need to last up to a minimum of five days (heatwave), which does not conform OECD (2004) guidelines of an acute toxicity test (48–72h). We propose future research to include a longer sublethal exposure to chlorpyrifos in combination with the occurrence of a heatwave, after which the organisms can be monitored for life history and physiological (energy budget, detoxification enzymes, etc.) responses (Tüzün & Stoks, 2020; Dinh Van et al., 2016). So far, this was beyond the aim of our study.

5 | CONCLUSIONS, POTENTIAL IMPLICATIONS AND FUTURE DIRECTIONS

We showed evolved chlorpyrifos resistance in urban water flea populations, similar to observations in one other nontarget organisms (damselflies, Tüzün et al., 2015). These results corroborate the evidence that aquatic nontarget organisms are likely adapting to pesticide pollution in urban areas, which enters ponds via surface run-off and aerial depositions. While we only tested resistance to chlorpyrifos, it is likely urban water fleas adapt to pesticides of the organophosphate and carbamate group (commonly applied in municipal and business districts), given they have similar modes of action and cross-tolerance to different pesticides and other pollutants has been demonstrated in water fleas, wood frogs and fish (Bendis & Relyea, 2016a; Hua et al., 2014; Oziolor et al., 2016). Such evolved resistance to multiple pesticides, across multiple organisms in the aquatic food web, could significantly modulate genetic and species diversity in urban ponds, as well as associated ecosystem functions. For example, evolved chlorpyrifos resistance in *Daphnia pulex*

prevented the development of a noxious algal bloom and impacted amphibian survivorship (Bendis & Relyea, 2016b). Although impacts of field exposures to pesticides seem predictable from ecotoxicological laboratory exposures (van der Hoeven & Gerritsen, 1997), future research on toxicant resistance in urban freshwater communities should specifically include targeted transplant experiments of focal species and communities (Brans et al., 2020). This enables to assess *in situ* to what extent evolution in multiple species in response to a multi-stressor (warming, pollution, disturbance) anthropogenic environment leads to costs of adaptation or can ultimately stabilize ecosystem properties (water clarity, biodiversity), which will feed back to society via socio-eco-evolutionary feedbacks (Des Roches et al., 2020). Finally, we propose to test the impact of new pesticides allowed in organic agriculture, such as pyrethrins and pyrethroids, on nontarget species, as these pesticides are increasingly popular (also in community gardening efforts in cities) and a transition from synthetic conventional pesticides to pesticides of more natural origins is currently promoted under the current EU Common Agricultural Policy (2014–2020).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

R script and data are currently available on GitHub (https://github.com/MFajgenblat/brglmm-chlorpyrifos). Data are additionally stored on Dryad.

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REFERENCES

Alberti, M., Palkovacs, E. P., Roches, S. D., De Meester, L., Brans, K. I., Govaert, L., Grimm, N. B., Harris, N. C., Hendry, A. P., Schell, C. J., Szulkin, M., Munshi-South, J., Urban, M. C., & Verrelli, B. C. (2020). The complexity of urban eco-evolutionary dynamics. *BioScience*, *70*(9), 772–793. https://doi.org/10.1093/biosci/biaa079

Allinson, G., Zhang, P., Bui, A. D., Allinson, M., Rose, G., Marshall, S., & Pettigrove, V. (2015). Pesticide and trace metal occurrence and aquatic benchmark exceedances in surface waters and sediments of urban wetlands and retention ponds in Melbourne, Australia. *Environmental Science and Pollution Research*, *22*(13), 10214–10226. https://doi.org/10.1007/s11356-015-4206-3

Aronson, M. F. J., La Sorte, F. A., Nilon, C. H., Katti, M., Goddard, M. A., Lepczyk, C. A., Warren, P. S., Williams, N. S. G., Cilliers, S., Clarkson, B., Dobbs, C., Dolan, R., Hedblom, M., Klotz, S., Kooijmans, J. L., Kühn, I., MacGregor-Fors, I., McDonnell, M., Mörtberg, U., … Winter, M. (2014). A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers.

Proceedings of the Royal Society B: Biological Sciences, *281*(1780), 20133330. https://doi.org/10.1098/rspb.2013.3330

Aronson, M. F. J., Nilon, C. H., Lepczyk, C. A., Parker, T. S., Warren, P. S., Cilliers, S. S., Goddard, M. A., Hahs, A. K., Herzog, C., Katti, M., La Sorte, F. A., Williams, N. S. G., & Zipperer, W. (2016). Hierarchical filters determine community assembly of urban species pools. *Ecology*, *97*(11), 2952–2963. https://doi.org/10.1002/ecy.1535

Augusiak, J., & van den Brink, P. J. (2016). The influence of insecticide exposure and environmental stimuli on the movement behaviour and dispersal of a freshwater isopod. *Ecotoxicology*, *25*(7), 1338–1352. https://doi.org/10.1007/s10646-016-1686-y

Barbosa, M., Inocentes, N., Soares, A. M. V. M., & Oliveira, M. (2017). Synergy effects of fluoxetine and variability in temperature lead to proportionally greater fitness costs in *Daphnia*: A multigenerational test. *Aquatic Toxicology*, *193*, 268–275. https://doi.org/10.1016/j.aquatox.2017.10.017

Bendis, R. J., & Relyea, R. A. (2014). Living on the edge: Populations of two zooplankton species living closer to agricultural fields are more resistant to a common insecticide. *Environmental Toxicology and Chemistry*, *33*(12), 2835–2841. https://doi.org/10.1002/etc.2749

Bendis, R. J., & Relyea, R. A. (2016a). If you see one, have you seen them all?: Community-wide effects of insecticide cross-resistance in zooplankton populations near and far from agriculture. *Environmental Pollution*, *215*, 234–246. https://doi.org/10.1016/j.envpol.2016.05.020

Bendis, R. J., & Relyea, R. A. (2016b). Wetland defense: naturally occurring pesticide resistance in zooplankton populations protects the stability of aquatic communities. *Oecologia*, *181*(2), 487–498. https://doi.org/10.1007/s00442-016-3574-9

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., Engelen, J. M. T., Souffreau, C., & De Meester, L. (2018). Urban hot-tubs: Local urbanization has profound effects on average and extreme temperatures in ponds. *Landscape and Urban Planning*, *176*, 22–29. https://doi.org/10.1016/j.landurbplan.2018.03.013

Brans, K. I., Govaert, L., & De Meester, L. (2020). Evolutionary Dynamics of Metacommunities in Urbanized Landscapes. In M. Szulkin, J. Munshi-South, & A. Charmantier (Eds.), *Urban Evolutionary Biology* (pp. 175–196). Oxford University Press.

Brans, K. I., Govaert, L., Engelen, J. M. T., Gianuca, A. T., Souffreau, C., & De Meester, L. (2017). Eco-evolutionary dynamics in urbanized landscapes: Evolution, species sorting and the change in zooplankton body size along urbanization gradients. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1712), 20160030. https://doi.org/10.1098/rstb.2016.0030

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

Brans, K. I., Stoks, R., & De Meester, L. (2018). Urbanization drives genetic differentiation in physiology and structures the evolution of pace-of-life syndromes in the water flea *Daphnia magna*. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1883), 20180169. https://doi.org/10.1098/rspb.2018.0169

Brock, T. C. M., & Van Wijngaarden, R. P. A. (2012). Acute toxicity tests with *Daphnia magna*, *Americamysis bahia*, *Chironomus riparius* and *Gammarus pulex* and implications of new EU requirements for the aquatic effect assessment of insecticides. *Environmental Science and Pollution Research*, *19*(8), 3610–3618. https://doi.org/10.1007/s11356-012-0930-0

Cáceres, T., He, W., Naidu, R., & Megharaj, M. (2007). Toxicity of chlorpyrifos and TCP alone and in combination to *Daphnia carinata*: The influence of microbial degradation in natural water. *Water Research*, *41*(19), 4497–4503. https://doi.org/10.1016/j.watres.2007.06.025

Carpenter, B., Gelman, A., Hoffman, M., Lee, D., Goodrich, B., Betancourt, M., Brubaker, M. A., Guo, J., Li, P., & Riddell, A. (2017). Stan: A Probabilistic Programming Language. *Journal of Statistical Software, Articles*, 76(1), 1–32. https://doi.org/10.18637/jss.v076.i01

Cheptou, P.-O., Hargreaves, A. L., Bonte, D., & Jacquemyn, H. (2017). Adaptation to fragmentation: evolutionary dynamics driven by human influences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1712), 20160037. https://doi.org/10.1098/rstb.2016.0037

Chislock, M. F., Sarnelle, O., Jernigan, L. M., & Wilson, A. E. (2013). Do high concentrations of microcystin prevent *Daphnia* control of phytoplankton? *Water Research*, 47(6), 1960–1971. https://doi.org/10.1016/j.watres.2012.12.038

Coors, A., Vanoverbeke, J., De Bie, T., & De Meester, L. (2009). Land use, genetic diversity and toxicant tolerance in natural populations of *Daphnia magna*. *Aquatic Toxicology*, 95(1), 71–79. https://doi.org/10.1016/j.aquatox.2009.08.004

Cowgill, U. M., Williams, D. M., & Esquivel, J. B. (1984). Effects of maternal nutrition on fat content and longevity of neonates of *Daphnia magna*. *Journal of Crustacean Biology*, 4(2), 173–190. https://doi.org/10.2307/1548014

Cuenca Cambronero, M., Marshall, H., De Meester, L., Davidson, T. A., Beckerman, A. P., & Orsini, L. (2018). Predictability of the impact of multiple stressors on the keystone species *Daphnia*. *Scientific Reports*, 8(1), 17572. https://doi.org/10.1038/s41598-018-35861-y

Cuppen, J. G. M., Crum, S. J. H., Van Den Heuvel, H. H., Smidt, R. A., & van den Brink, P. J. (2002). Effects of a mixture of two insecticides in freshwater microcosms: I. Fate of chlorpyrifos and lindane and responses of macroinvertebrates. *Ecotoxicology*, 11(3), 165–180. https://doi.org/10.1023/A:1015470731330

Dallimer, M., Rouquette, J. R., Skinner, A. M. J., Armsworth, P. R., Maltby, L. M., Warren, P. H., & Gaston, K. J. (2012). Contrasting patterns in species richness of birds, butterflies and plants along riparian corridors in an urban landscape. *Diversity and Distributions*, 18(8), 742–753. https://doi.org/10.1111/j.1472-4642.2012.00891.x

De Coen, W. M., & Janssen, C. R. (2003). The missing biomarker link: Relationships between effects on the cellular energy allocation biomarker of toxicant-stressed *Daphnia magna* and corresponding population characteristics. *Environmental Toxicology and Chemistry*, 22(7), 1632–1641. https://doi.org/10.1002/ETC.5620220727

De Meester, L., Brans, K. I., Govaert, L., Souffreau, C., Mukherjee, S., Vanvelk, H., Korzeniowski, K., Kilsdonk, L., Decaestecker, E., Stoks, R., & Urban, M. C. (2019). Analysing ecoevolutionary dynamics—The challenging complexity of the real world. *Functional Ecology*, 33(1), 43–59. https://doi.org/10.1111/1365-2435.13261

Des Roches, S., Brans, K. I., Lambert, M. R., Rivkin, L. R., Savage, A. M., Schell, C. J., & Alberti, M. (2020). Socio-eco-evolutionary dynamics in cities. *Evolutionary Applications*, 14(1), 248–267. https://doi.org/10.1111/eva.13065

De Saeger, S., Oosterlynck, P., & Paelinckx, D. (2017). *The Biological Valuation Map (BVM): a field-driven survey of land cover and vegetation in the Flemish Region of Belgium*.

Diamond, S. E., Chick, L., Perez, A., Strickler, S. A., & Martin, R. A. (2017). Rapid evolution of ant thermal tolerance across an urban-rural temperature cline. *Biological Journal of the Linnean Society*, 121(2), 248–257. https://doi.org/10.1093/BIOLINNEAN/BLW047

Donihue, C. M., & Lambert, M. R. (2015). Adaptive evolution in urban ecosystems. *Ambio*, 44(3), 194–203. https://doi.org/10.1007/s13280-014-0547-2

Echeverri-Jaramillo, G., Jaramillo-Colorado, B., Sabater-Marco, C., & Castillo-López, M. Á. (2020). Acute toxicity of chlorpyrifos and its metabolite 3,5,6-trichloro-2-pyridinol alone and in combination using a battery of bioassays. *Environmental Science and Pollution Research*, 27(26), 32770–32778. https://doi.org/10.1007/s11356-020-09392-x

Ferrario, C., Parolini, M., De Felice, B., Villa, S., & Finizio, A. (2018). Linking sub-individual and supra-individual effects in *Daphnia magna* exposed to sub-lethal concentration of chlorpyrifos. *Environmental Pollution*, 235, 411–418. https://doi.org/10.1016/j.envpol.2017.12.113

Gaizick, L., Gupta, G., & Bass, E. (2001). Toxicity of chlorpypyrifos to *Rana pipiens* embryos. *Bulletin of Environmental Contamination and Toxicology*, 66(3), 386–391. https://doi.org/10.1007/s001280017

Geerts, A. N., Vanoverbeke, J., Vanschoenwinkel, B., Van Doorslaer, W., Feuchtmayr, H., Atkinson, D., Moss, B., Davidson, T. A., Sayer, C. D., & De Meester, L. (2015). Rapid evolution of thermal tolerance in the water flea *Daphnia*. *Nature Climate Change*, 5(7), 665–668. https://doi.org/10.1038/nclimate2628

Gelman, A., Carlin, J. B., Stern, H. S., Dunson, D. B., Vehtari, A., & Rubin, D. B. (2014). *Bayesian Data Analysis*. 3rd ed. Chapman and Hall/CRC. https://doi.org/10.1201/b16018

Gianuca, A. T., Pantel, J. H., & De Meester, L. (2016). Disentangling the effect of body size and phylogenetic distances on zooplankton top-down control of algae. *Proceedings of the Royal Society B: Biological Sciences*, 283(1828), 20160487. https://doi.org/10.1098/rspb.2016.0487

Giddings, J. M., Williams, W. M., Solomon, K. R., & Giesy, J. P. (2014). Risks to aquatic organisms from use of chlorpyrifos in the United States. *Reviews of Environmental Contamination and Toxicology*, 231, 119–162. https://doi.org/10.1007/978-3-319-03865-0_5

Grimm, N. B., Foster, D., Groffman, P., Grove, J. M., Hopkinson, C. S., Nadelhoffer, K. J., Pataki, D. E., & Peters, D. P. (2008). The changing landscape: ecosystem responses to urbanization and pollution across climatic and societal gradients. *Frontiers in Ecology and the Environment*, 6(5), 264–272. https://doi.org/10.1890/070147

Hasenbein, S., Peralta, J., Lawler, S. P., & Connon, R. E. (2017). Environmentally relevant concentrations of herbicides impact non-target species at multiple sublethal endpoints. *Science of the Total Environment*, 607–608, 733–743. https://doi.org/10.1016/j.scitotenv.2017.06.270

Hendry, A. P., Gotanda, K. M., & Svensson, E. I. (2017). Human influences on evolution, and the ecological and societal consequences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1712), 20160028. https://doi.org/10.1098/rstb.2016.0028

Heudorf, U., Angerer, J., & Drexler, H. (2004). Current internal exposure to pesticides in children and adolescents in Germany: Urinary levels of metabolites of pyrethroid and organophosphorus insecticides. *International Archives of Occupational and Environmental Health*, 77(1), 67–72. https://doi.org/10.1007/s00420-003-0470-5

Holmstrup, M., Bindesbøl, A. M., Oostingh, G. J., Duschl, A., Scheil, V., Köhler, H. R., Loureiro, S., Soares, A. M. V. M., Ferreira, A. L. G., Kienle, C., Gerhardt, A., Laskowski, R., Kramarz, P. E., Bayley, M., Svendsen, C., & Spurgeon, D. J. (2010). Interactions between effects of environmental chemicals and natural stressors: A review. *Science of the Total Environment*, 408(18), 3746–3762. https://doi.org/10.1016/j.scitotenv.2009.10.067

Hooper, M. J., Ankley, G. T., Cristol, D. A., Maryoung, L. A., Noyes, P. D., & Pinkerton, K. E. (2013). Interactions between chemical and climate stressors: A role for mechanistic toxicology in assessing climate change risks. *Environmental Toxicology and Chemistry*, 32(1), 32–48. https://doi.org/10.1002/etc.2043

Hua, J., Jones, D. K., & Relyea, R. A. (2014). Induced tolerance from a sublethal insecticide leads to cross-tolerance to other insecticides. *Environmental Science and Technology*, 48(7), 4078–4085. https://doi.org/10.1021/es500278f

Huang, X., Cui, H., & Duan, W. (2020). Ecotoxicity of chlorpyrifos to aquatic organisms: A review. *Ecotoxicology and Environmental Safety*, 200, 110731. https://doi.org/10.1016/j.ecoenv.2020.110731

IBZ (2021). *Chiffres de la population par province et par commune, a la date du 1er Janvier 2021* (Flandres, Belgique).

Jansen, M., Coors, A., Vanoverbeke, J., Schepens, M., De Voogt, P., De Schamphelaere, K. A. C., & De Meester, L. (2015). Experimental evolution reveals high insecticide tolerance in *Daphnia* inhabiting farmland ponds. *Evolutionary Applications*, 8(5), 442–453. https://doi.org/10.1111/eva.12253

Jeon, J., Kretschmann, A., Escher, B. I., & Hollender, J. (2013). Characterization of acetylcholinesterase inhibition and energy allocation in *Daphnia magna* exposed to carbaryl. *Ecotoxicology and Environmental Safety*, 98, 28–35. https://doi.org/10.1016/j.ecoenv.2013.09.033

Johnson, M. T. J., & Munshi-South, J. (2017). Evolution of life in urban environments. *Science*, 358(6363), eaam8327. https://doi.org/10.1126/science.aam8327

Juárez, M. A., & Steel, M. F. J. (2010). Model-based clustering of non-gaussian panel data based on skew-t distributions. *Journal of Business and Economic Statistics*, 28(1), 52–66. https://doi.org/10.1198/jbes.2009.07145

Julien, R., Adamkiewicz, G., Levy, J. I., Bennett, D., Nishioka, M., & Spengler, J. D. (2008). Pesticide loadings of select organophosphate and pyrethroid pesticides in urban public housing. *Journal of Exposure Science and Environmental Epidemiology*, 18(2), 167–174. https://doi.org/10.1038/sj.jes.7500576

Kamdem, C., Fouet, C., Gamez, S., & White, B. J. (2017). Pollutants and insecticides drive local adaptation in African Malaria mosquitoes. *Molecular Biology and Evolution*, 34(5), 1261–1275. https://doi.org/10.1093/molbev/msx087

Kay, M. (2020). *tidybayes: Tidy data and geoms for Bayesian models. R package version 2.2.0*. https://doi.org/10.5281/zenodo.1308151

Kersting, K., & van Wijngaarden, R. (1992). Effects of chlorpyrifos on a microecosystem. *Environmental Toxicology and Chemistry*, 11(3), 365–372. https://doi.org/10.1002/etc.5620110310

Knillmann, S., Stampfli, N. C., Noskov, Y. A., Beketov, M. A., & Liess, M. (2012). Interspecific competition delays recovery of *Daphnia* spp. populations from pesticide stress. *Ecotoxicology*, 21(4), 1039–1049. https://doi.org/10.1007/s10646-012-0857-8

Knillmann, S., Stampfli, N. C., Noskov, Y. A., Beketov, M. A., & Liess, M. (2013). Elevated temperature prolongs long-term effects of a pesticide on *Daphnia* spp. due to altered competition in zooplankton communities. *Global Change Biology*, 19(5), 1598–1609. https://doi.org/10.1111/gcb.12151

Li, Y., Xu, J., Zhong, D., Zhang, H., Yang, W., Zhou, G., Su, X., Wu, Y., Wu, K., Cai, S., Yan, G., & Chen, X. G. (2018). Evidence for multiple-insecticide resistance in urban *Aedes albopictus* populations in southern China. *Parasites and Vectors*, 11(1), 4. https://doi.org/10.1186/s13071-017-2581-y

López-Mancisidor, P., Carbonell, G., Fernández, C., & Tarazona, J. V. (2008). Ecological impact of repeated applications of chlorpyrifos on zooplankton community in mesocosms under Mediterranean conditions. *Ecotoxicology*, 17(8), 811–825. https://doi.org/10.1007/s10646-008-0239-4

LRD. (2013). *Large-scale Reference Catabase, an object-oriented reference map of Flanders*. Available via https://overheid.vlaanderen.be/en/producten-diensten/large-scale-reference-database-lrd

Macoris, M. D. L. G., Andrighetti, M. T. M., Takaku, L., Glasser, C. M., Garbeloto, V. C., & Bracco, J. E. (2003). Resistance of *Aedes aegypti* from the State of São Paulo, Brazil, to Organophosphates Insecticides. *Memorias do Instituto Oswaldo Cruz*, 98(5), 703–708. https://doi.org/10.1590/S0074-02762003000500020

Major, K. M., Weston, D. P., Lydy, M. J., Wellborn, G. A., & Poynton, H. C. (2018). Unintentional exposure to terrestrial pesticides drives widespread and predictable evolution of resistance in freshwater crustaceans. *Evolutionary Applications*, 11(5), 748–761. https://doi.org/10.1111/eva.12584

Martin, R. A., Chick, L. D., Yilmaz, A. R., & Diamond, S. E. (2019). Evolution, not transgenerational plasticity, explains the adaptive divergence of acorn ant thermal tolerance across an urban–rural temperature cline. *Evolutionary Applications*, 12(8), 1678–1687. http://dx.doi.org/10.1111/eva.12826

McKelvey, W., Jacobson, J. B., Kass, D., Barr, D. B., Davis, M., Calafat, A. M., & Aldous, K. M. (2013). Population-based biomonitoring of exposure to organophosphate and pyrethroid pesticides in New York city. *Environmental Health Perspectives*, 121(11–12), 1349–1356. https://doi.org/10.1289/ehp.1206015

Meftaul, I. M. D., Venkateswarlu, K., Dharmarajan, R., Annamalai, P., & Megharaj, M. (2020). Pesticides in the urban environment: A potential threat that knocks at the door. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2019.134612

Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L. F., Backeljau, T., Bonte, D., Brans, K. I., Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J. M. T., Fontaneto, D., Gianuca, A. T., Govaert, L., Hendrickx, F., Higuti, J., Lens, L., Martens, K., … Van Dyck, H. (2018). Body-size shifts in aquatic and terrestrial urban communities. *Nature*, 558(7708), 113–116. https://doi.org/10.1038/s41586-018-0140-0

Messiaen, M., De Schamphelaere, K. A. C., Muyssen, B. T. A., & Janssen, C. R. (2010). The micro-evolutionary potential of *Daphnia magna* population exposed to temperature and cadmium stress. *Ecotoxicology and Environmental Safety*, 73(6), 1114–1122. https://doi.org/10.1016/j.ecoenv.2010.05.006

Miles, L. S., Rivkin, L. R., Johnson, M. T. J., Munshi-South, J., & Verrelli, B. C. (2019). Gene flow and genetic drift in urban environments. *Molecular Ecology*, 28(18), 4138–4151. https://doi.org/10.1111/mec.15221

Moe, S. J., De Schamphelaere, K., Clements, W. H., Sorensen, M. T., van den Brink, P. J., & Liess, M. (2013). Combined and interactive effects of global climate change and toxicants on populations and communities. *Environmental Toxicology and Chemistry*, 32(1), 49–61. https://doi.org/10.1002/etc.2045

Moore, M. T., Huggett, D. B., Gillespie, W. B., Rodgers, J. H., & Cooper, C. M. (1998). Comparative toxicity of chlordane, chlorpyrifos, and aldicarb to four aquatic testing organisms. *Archives of Environmental Contamination and Toxicology*, 34(2), 152–157. https://doi.org/10.1007/s002449900299

Munshi-South, J., & Richardson, J. L. (2020). Landscape genetic approaches to understanding movement and gene flow in cities. In M. Szulkin, J. Munshisouth, A. Charmantier (Eds.), *Urban Evolutionary Biology* (1st ed., pp. 55–73). Oxford University Press.

Naddy, R. B., Johnson, K. A., & Klaine, S. J. (2009). Response of *Daphnia magna* to pulsed exposures of chlorpyrifos. *Environmental Toxicology and Chemistry*, 19(2), 423–431. https://doi.org/10.1002/etc.5620190223

Noyes, P. D., McElwee, M. K., Miller, H. D., Clark, B. W., Van Tiem, L. A., Walcott, K. C., Erwin, K. N., & Levin, E. D. (2009). The toxicology of climate change: Environmental contaminants in a warming world. *Environment International*, 35(6), 971–986. Elsevier Ltd. https://doi.org/10.1016/j.envint.2009.02.006

OECD. (2004). OECD (Organisation for Economic Cooperation and Development) guidelines for the testing of chemicals. Guide- line 202: Daphnia sp., Acute Immobilisation Test. Adopted April 2004, Paris, pp. 21.

Op de Beeck, L., Verheyen, J., Olsen, K., & Stoks, R. (2017). Negative effects of pesticides under global warming can be counteracted by a higher degradation rate and thermal adaptation. *Journal of Applied Ecology*, 54(6), 1847–1855. https://doi.org/10.1111/1365-2664.12919

Oziolor, E. M., Dubansky, B., Burggren, W. W., & Matson, C. W. (2016). Cross-resistance in Gulf killifish (*Fundulus grandis*) populations resistant to dioxin-like compounds. *Aquatic Toxicology*, 175, 222–231. https://doi.org/10.1016/j.aquatox.2016.03.019

Oziolor, E. M., & Matson, C. W. (2018). Adaptation in polluted waters: Lessons from killifish. In W. Burggren, & B. Dubansky (Eds.), *Development and Environment* (pp. 355–375). Springer International Publishing. https://doi.org/10.1007/978-3-319-75935-7_14

Palma, P., Palma, V. L., Fernandes, R. M., Soares, A. M. V. M., & Barbosa, I. R. (2008). Acute toxicity of atrazine, endosulfan sulphate and chlorpyrifos to *Vibrio fischeri, Thamnocephalus platyurus* and *Daphnia magna*, relative to their concentrations in surface waters from the Alentejo region of Portugal. *Bulletin of Environmental Contamination and Toxicology*, 81(5), 485–489. https://doi.org/10.1007/s00128-008-9517-3

Palma, P., Palma, V. L., Matos, C., Fernandes, R. M., Bohn, A., Soares, A. M. V. M., & Barbosa, I. R. (2009). Assessment of the pesticides atrazine, endosulfan sulphate and chlorpyrifos for juvenoid-related endocrine activity using *Daphnia magna. Chemosphere*, 76(3), 335–340. https://doi.org/10.1016/j.chemosphere.2009.03.059

Palumbi, S. R. (2001). Humans as the world's greatest evolutionary force. *Science*, 293(5536), 1786–1790. https://doi.org/10.1126/science.293.5536.1786

Pang, Y., MacIntosh, D. L., Camann, D. E., & Ryan, P. B. (2002). Analysis of aggregate exposure to chlorpyrifos in the NHEXAS-Maryland investigation. *Environmental Health Perspectives*, 110(3), 235–240. https://doi.org/10.1289/ehp.02110235

Parris, K. M. (2016). *Ecology of Urban Environments*. John Wiley & Sons Ltd.

Pereira Lima, J. B., Pereira Da-Cunha, M., Carneiro Da Silva, R., Ribeiro Galardo, A. K., Da Silva Soares, S. D., Aparecida Braga, I., Lima, J. B. P., & Valle, D. (2003). Resistance of *Aedes aegypti* to organophosphates in several municipalities in the state of Rio de Janeiro and Espírito Santo, Brazil. *American Journal of Tropical Medicine and Hygiene*, 68(3), 329–333. https://doi.org/10.4269/ajtmh.2003.68.329

Pérez, S., Rial, D., & Beiras, R. (2015). Acute toxicity of selected organic pollutants to saltwater (mysid *Siriella armata*) and freshwater (cladoceran *Daphnia magna*) ecotoxicological models. *Ecotoxicology*, 24(6), 1229–1238. https://doi.org/10.1007/s10646-015-1489-6

Piano, E., De Wolf, K., Bona, F., Bonte, D., Bowler, D. E., Isaia, M., Lens, L., Merckx, T., Mertens, D., van Kerckvoorde, M., De Meester, L., & Hendrickx, F. (2017). Urbanization drives community shifts towards thermophilic and dispersive species at local and landscape scales. *Global Change Biology*, 23(7), 2554–2564. https://doi.org/10.1111/gcb.13606

Piano, E., Souffreau, C., Merckx, T., Baardsen, L. F., Backeljau, T., Bonte, D., Brans, K. I., Cours, M., Dahirel, M., Debortoli, N., Decaestecker, E., De Wolf, K., Engelen, J. M. T., Fontaneto, D., Gianuca, A. T., Govaert, L., Hanashiro, F. T. T., Higuti, J., Lens, L., ... Hendrickx, F. (2020). Urbanization drives cross-taxon declines in abundance and diversity at multiple spatial scales. *Global Change Biology*, 26(3), 1196–1211. https://doi.org/10.1111/gcb.14934

R Core Team (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Raymundo, L. B., Rocha, O., Moreira, R. A., Miguel, M., & Daam, M. A. (2019). Sensitivity of tropical cladocerans to chlorpyrifos and other insecticides as compared to their temperate counterparts. *Chemosphere*, 220, 937–942. https://doi.org/10.1016/j.chemosphere.2019.01.005

Romero, A., & Anderson, T. D. (2016). High levels of resistance in the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), to neonicotinoid insecticides. *Journal of Medical Entomology*, 53(3), 727–731. https://doi.org/10.1093/jme/tjv253

Rumschlag, S. L., Mahon, M. B., Hoverman, J. T., Raffel, T. R., Carrick, H. J., Hudson, P. J., & Rohr, J. R. (2020). Consistent effects of pesticides on community structure and ecosystem function in freshwater systems. *Nature Communications*, 11(1), 1–9. https://doi.org/10.1038/s41467-020-20192-2

Sakai, M. (2002). Determination of pesticides and chronic test with *Daphnia magna* for rainwater samples. *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes*, 37(3), 247–254. https://doi.org/10.1081/PFC-120003102

Sancho, E., Villarroel, M. J., Andreu, E., & Ferrando, M. D. (2009). Disturbances in energy metabolism of *Daphnia magna* after exposure to tebuconazole. *Chemosphere*, 74(9), 1171–1178. https://doi.org/10.1016/j.chemosphere.2008.11.076

Schulz, R. (2004). Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: a review. *Journal of Environmental Quality*, 33(2), 419–448. https://doi.org/10.2134/jeq2004.4190

Silva, E., Martins, C., Pereira, A. S., Loureiro, S., & Cerejeira, M. J. (2018). Toxicity prediction and assessment of an environmentally realistic pesticide mixture to *Daphnia magna* and *Raphidocelis subcapitata*. *Ecotoxicology*, 27(7), 956–967. https://doi.org/10.1007/s10646-018-1938-0

Simpson, A. M., Jeyasingh, P. D., & Belden, J. B. (2017). Assessment of biochemical mechanisms of tolerance to chlorpyrifos in ancient and contemporary *Daphnia pulicaria* genotypes. *Aquatic Toxicology*, 193, 122–127. https://doi.org/10.1016/j.aquatox.2017.10.012

Sokolova, I. M. (2013). Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integrative and Comparative Biology*, 53(4), 597–608. https://doi.org/10.1093/icb/ict028

Song, Y., Chen, M., & Zhou, J. (2017). Effects of three pesticides on superoxide dismutase and glutathione-S-transferase activities and reproduction of *Daphnia magna*. *Archives of Environmental Protection*, 43(1), 80–86. https://doi.org/10.1515/aep-2017-0010

Stan Development Team (2020). RStan: The R interface to Stan. R package version 2.21.2. http://mc-stan.org/

Stehle, S., & Schulz, R. (2015). Agricultural insecticides threaten surface waters at the global scale. *Proceedings of the National Academy of Sciences of the United States of America*, 112(18), 5750–5755. https://doi.org/10.1073/pnas.1500232112

Theys, C., Verheyen, J., Tüzün, N., & Stoks, R. (2020). Higher mean and fluctuating temperatures jointly determine the impact of the pesticide chlorpyrifos on the growth rate and leaf consumption of a freshwater isopod. *Chemosphere*, 273, 128528. https://doi.org/10.1016/j.chemosphere.2020.128528

Tüzün, N., Debecker, S., Op de Beeck, L., & Stoks, R. (2015). Urbanisation shapes behavioural responses to a pesticide. *Aquatic Toxicology*, 163, 81–88. https://doi.org/10.1016/J.AQUATOX.2015.04.002

Tüzün, N., & Stoks, R. (2020). Lower bioenergetic costs but similar immune responsiveness under a heat wave in urban compared to rural damselflies. *Evolutionary Applications*, 14(1), 24–35. https://doi.org/10.1111/eva.13041

van den Brink, P. J., Boxall, A. B. A., Maltby, L., Brooks, B. W., Rudd, M. A., Backhaus, T., Spurgeon, D., Verougstraete, V., Ajao, C., Ankley, G. T., Apitz, S. E., Arnold, K., Brodin, T., Cañedo-Argüelles, M., Chapman, J., Corrales, J., Coutellec, M.-A., Fernandes, T. F., Fick, J., ... van Wensem, J. (2018). Toward sustainable environmental quality: Priority research questions for Europe. *Environmental Toxicology and Chemistry*, 37(9), 2281–2295. Wiley Blackwell. https://doi.org/10.1002/etc.4205

van den Brink, P. J., van Donk, E., Gylstra, R., Crum, S. J. H., & Brock, T. C. M. (1995). Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. *Chemosphere*, 31(5), 3181–3200. https://doi.org/10.1016/0045-6535(95)00180-G

van der Hoeven, N., & Gerritsen, A. A. M. (1997). Effects of chlorpyrifos on individuals and populations of *Daphnia pulex* in the laboratory and field. *Environmental Toxicology and Chemistry*, 16(12), 2438–2447. https://doi.org/10.1002/etc.5620161202

Van Dinh, K., Janssens, L., Debecker, S., De Jonge, M., Lambret, P., Nilsson-Örtman, V., Bervoets, L., & Stoks, R. (2013). Susceptibility to a metal under global warming is shaped by thermal adaptation

along a latitudinal gradient. *Global Change Biology*, 19(9), 2625–2633. https://doi.org/10.1111/gcb.12243

Van Dinh, K., Janssens, L., & Stoks, R. (2016). Exposure to a heat wave under food limitation makes an agricultural insecticide lethal: a mechanistic laboratory experiment. *Global Change Biology*, 22(10), 3361–3372. https://doi.org/10.1111/gcb.13415

Vehtari, A., Gelman, A., Simpson, D., Carpenter, B., & Bürkner, P.-C. (2021). Rank-Normalization, folding, and localization: An improved Rˆ for assessing convergence of MCMC (with Discussion). *Bayesian Analysis*, 16(2). http://dx.doi.org/10.1214/20-ba1221

Verheyen, J., Delnat, V., & Stoks, R. (2019). Increased daily temperature fluctuations overrule the ability of gradual thermal evolution to offset the increased pesticide toxicity under global warming. *Environmental Science and Technology*, 53(8), 4600–4608. https://doi.org/10.1021/acs.est.8b07166

Vijftigschildt, R. A. N., Olsthoorn, C. S. M., & Loorij, T. P. J. (2005). *Collecting information on the use of plant protection products outside agriculture.*

Wang, L., Ye, W., Zhou, S., Lin, K., Zhao, M., & Liu, W. (2009). Acute and chronic toxicity of organophosphate monocrotophos to *Daphnia magna*. *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes*, 44(1), 38–43. https://doi.org/10.1080/03601230802519611

Whitehead, A., Pilcher, W., Champlin, D., & Nacci, D. (2012). Common mechanism underlies repeated evolution of extreme pollution tolerance. *Proceedings of the Royal Society B: Biological Sciences*, 279(1728), 427–433. https://doi.org/10.1098/rspb.2011.0847

Wirgin, I., Roy, N. K., Loftus, M., Chambers, R. C., Franks, D. G., & Hahn, M. E. (2011). Mechanistic basis of resistance to PCBs in Atlantic tomcod from the Hudson River. *Science (New York, N.Y.)*, 331(6022), 1322–1325. https://doi.org/10.1126/science.1197296

Wittmer, I. K., Bader, H. P., Scheidegger, R., Singer, H., Lück, A., Hanke, I., Carlsson, C., & Stamm, C. (2010). Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters. *Water Research*, 44(9), 2850–2862. https://doi.org/10.1016/j.watres.2010.01.030

Wood, B., & Stark, J. D. (2002). Acute toxicity of drainage ditch water from a Washington State cranberry-growing region to *Daphnia pulex* in laboratory bioassays. *Ecotoxicology and Environmental Safety*, 53(2), 273–280. https://doi.org/10.1006/eesa.2002.2210

Wouters, H., De Ridder, K., Poelmans, L., Willems, P., Brouwers, J., Hosseinzadehtalaei, P., Tabari, H., Vanden Broucke, S., van Lipzig, N. P. M., & Demuzere, M. (2017). Heat stress increase under climate change twice as large in cities as in rural areas: A study for a densely populated midlatitude maritime region. *Geophysical Research Letters*, 44(17), 8997–9007. https://doi.org/10.1002/2017GL074889

Wu, X., & Appel, A. G. (2017). Insecticide resistance of several field-collected German cockroach (Dictyoptera: Blattellidae) strains. *Journal of Economic Entomology*, 110(3), 1203–1209. https://doi.org/10.1093/jee/tox072

Zafar, M. I., Van Wijngaarden, R. P. A., Roessink, I., & Van den Brink, P. J. (2011). Effects of time-variable exposure regimes of the insecticide chlorpyrifos on freshwater invertebrate communities in microcosms. *Environmental Toxicology and Chemistry*, 30(6), 1383–1394. https://doi.org/10.1002/etc.509

Zalizniak, L., & Nugegoda, D. (2006). Effect of sublethal concentrations of chlorpyrifos on three successive generations of *Daphnia carinata*. *Ecotoxicology and Environmental Safety*, 64(2), 207–214. https://doi.org/10.1016/j.ecoenv.2005.03.015

Zhu, F., Lavine, L., O'Neal, S., Lavine, M., Foss, C., & Walsh, D. (2016). Insecticide resistance and management strategies in urban ecosystems. *Insects*, 7(1), 2. MDPI AG, https://doi.org/10.3390/insects7010002

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