



Urbanisation shapes behavioural responses to a pesticide



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ABSTRACT

The degree of urbanisation is rapidly increasing worldwide. Due to anthropogenic impact, urban populations are exposed to higher levels of contaminants and higher temperatures. Despite this, urbanisation is a largely overlooked spatial component in ecotoxicology. We tested in a common garden rearing experiment whether replicated urban and rural populations of the damselfly *Coenagrion puella* differ in their vulnerability to sublethal levels of a widespread pesticide, chlorpyrifos, in terms of ecologically relevant behaviours (exploration behaviour, activity, boldness and food intake), and to what extent these patterns are affected by temperature (20 and 24 °C). Except boldness, all behaviours were affected by previous pesticide exposure. While the pesticide did not affect exploration behaviour at 20 °C, it was associated with increased exploration at 24 °C, which may reflect an increased toxicity of chlorpyrifos at higher temperatures. Importantly, rural and urban larvae showed consistently different, sometimes even opposite behavioural responses to pesticide exposure. When exposed to the pesticide, rural larvae decreased activity and food intake at both temperatures; urban larvae instead increased activity at both temperatures and only reduced food intake at the high temperature. This suggests that urban larvae were less affected by the pesticide, which would be consistent with a scenario of local adaptation to higher contaminant levels. Our results highlight that urbanisation may be an important factor to arrive at a spatially explicit ecological risk assessment, and may be an ignored reason why studies on the same species may generate widely different vulnerabilities to pesticides.

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1. Introduction

Despite increasing attention for spatially explicit ecological risk assessment (Van den Brink, 2008; Clements et al., 2012) and the rapidly increasing urbanisation levels worldwide (United Nations, 2014), urbanisation is a largely overlooked spatial component in ecotoxicology. Urbanisation is regarded as a major threat to ecological communities (McKinney, 2008), as ecosystems in urban areas suffer from a range of anthropogenic pressures, including substantial accumulation of contaminants in urban surface water (Gilliom, 2007). It is, however, unknown to what extent this generates local adaptation where urban populations are better adjusted to deal with contaminants than rural ones. Such patterns may be expected as many studies indicated patterns of local adaptation where populations experiencing higher contaminant levels evolved a lower vulnerability to these contaminants (reviewed in Medina et al., 2007; but see e.g. Crespi, 2000; Spitzer, 2006).

Due to regulatory programs, most freshwater organisms in developed countries are only rarely facing lethal levels of pesti-

cides; yet there is increasing concern that also sublethal pesticide levels may have profound effects (Köhler and Triebkorn, 2013), eventually leading to biodiversity loss (Beketov et al., 2013). One important type of response variables that may mediate negative effects of sublethal pesticide doses are changes in ecologically relevant behaviours (Weis et al., 2001; Dell'Omo, 2002; Zala and Penn, 2004; Desneux et al., 2007). Contaminants at sublethal doses have been shown to affect behaviours such as activity (e.g. Relyea and Mills, 2001), boldness (e.g. Brodin et al., 2013), and food intake (e.g. Zubrod et al., 2014) in a wide range of taxa. Maladaptive behavioural changes that have a direct effect on trophic relations, such as predator–prey interactions, can have major implications for both the prey (e.g. reduced survival, Janssens and Stoks, 2012) and for the predator (e.g. reduced growth, Campero et al., 2007), which may eventually propagate through the food web (reviewed in Rohr et al., 2006). Given that behavioural responses to contaminants may have a genetic base (e.g. Breckels and Neff, 2010), it may be expected that locally adapted urban and rural populations consistently differ in their behavioural responses to pesticides.

Besides contamination, higher temperatures are another key abiotic stressor associated with urbanisation, creating the so-called urban heat islands (Oke, 1973; Arnfield, 2003). Temperatures in urban areas have been recorded to be up to 8 °C warmer compared to rural areas (e.g. Rosenzweig et al., 2009; Maiheu et al.,

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2013). These two stressors usually do not act independently, as many contaminants are known to be more toxic at higher temperatures, which is of considerable concern in a warming world (Noyes et al., 2009; Moe et al., 2013). Urban ecosystems have therefore been proposed as suitable study systems with the potential to provide valuable insights into the effects of global warming in a contaminated world (Grimm et al., 2008; Youngsteadt et al., 2014). As urban populations are generally exposed to both contamination and temperature increases (Gilliom, 2007; George et al., 2007), these populations may develop local adaptations making them less vulnerable to the increased toxicity of pesticides at higher temperatures (for an example along a large-scale temperature gradient, see Dinh Van et al., 2013). Yet thermal adaptation along temperature gradients may not always mitigate the toxicity of a contaminant at higher temperatures (e.g. Janssens et al., 2014).

In this study, we tested in a common garden rearing experiment from the egg stage whether replicated urban and rural populations of an aquatic insect consistently differ in their vulnerability to sublethal levels of a widespread pesticide in terms of ecologically relevant behaviours, and to what extent these patterns are affected by temperature. As study organisms we used damselfly larvae, important intermediate predators in aquatic food webs (Johnson, 1991; Stoks and Córdoba-Aguilar, 2012) that are specifically vulnerable to global warming (Hassall and Thompson, 2008). We focused on following fitness-related behaviours (Smith and Blumstein, 2008): exploration behaviour, activity and food intake (in the presence and absence of predator cues), and boldness. As model pesticide, we chose chlorpyrifos (CPF), one of the most frequently used pesticides worldwide (Eaton et al., 2008). CPF has been shown to alter various behaviours in damselflies; moreover, these effects were often magnified at higher temperatures (Janssens and Stoks, 2013b,b; Dinh Van et al., 2014a,b).

2. Materials and methods

2.1. Study populations and rearing protocol

We sampled six populations of the damselfly *Coenagrion puella*, one of the most common damselflies in Central Europe (Askew, 1988), occupying ponds in urban as well as natural areas (Goertzen and Suhling, 2013). We selected three ponds from urban and three from rural areas in Flanders (Table 1). The selection of the ponds was carried out following a two-step procedure using geographic information system (GIS). In a first step, six plots (3 × 3 km) were selected to represent two urbanisation levels defined by the percentage of anthropogenic built-up areas: urban (>15%) and rural (<3%). In a second step, we selected in each plot a pond in a subplot (200 × 200 m) with the same urbanisation level. This ensured that both the direct environment (subplot) and the broader surroundings (plot) reflected the same urbanisation level. All ponds were shallow water bodies with abundant aquatic vegetation, and contained large aeshnid dragonfly larvae as predators. Note that the chosen rural populations were not directly embedded by cropland (distance to nearest cropland: 110–500 m), and instead were always near to forest (distance to forest: 0–40 m) and surrounded by a high percentage of woodland (35–95%). It is therefore unlikely that they were affected by agriculture (Declerck et al., 2006).

We collected between 9 and 11 mated females per population during July and August 2013, and transferred them to the laboratory. Females were placed individually in 200 ml plastic cups and provided with wet filter paper for oviposition. For the first 10 days after hatching, larvae were kept in groups at 22 °C in order to enhance survival (De Block and Stoks, 2003). Thereafter, larvae were allocated individually to plastic cups (200 ml) with aged tap water, and kept in temperature-controlled water baths (20 °C

or 24 °C) with a constant photoperiod of 14:10 h light/dark. Larvae were daily fed *Artemia nauplii* ad libitum (daily food ration: 212 ± 67 nauplii, mean ± SE, $n = 12$). We chose these temperatures as 20 °C reflects a common summer water temperature in ponds that the study species inhabits in Flanders, while temperatures of 24 °C are also encountered, yet much less frequently (Jessie Engelen, unpublished data). The 4 °C temperature difference also reflects the predicted temperature increase by 2100 as predicted under IPCC scenario RCP8.5 (IPCC, 2013). Moreover, a recent report shows that in Flanders (e.g. city of Ghent) the temperature in the urban centre is on average 3 °C (and up to 8 °C) warmer than the bordering countryside (Maiheu et al., 2013), indicating that the 4 °C temperature difference used in our study is within the expected temperature difference between urban and rural areas.

2.2. Experimental setup

To test for effects of pesticide exposure, temperature and the urbanisation level of the pond of origin on the four behavioural traits (exploration behaviour, activity, food intake and boldness) of the larvae, we set up a full factorial common garden experiment with eight treatment combinations: two pesticide treatments (chlorpyrifos/control), two temperature treatments (20/24 °C), and two levels of urbanisation (urban/rural, each represented by three populations). After being randomly assigned to a rearing temperature of 20 °C or 24 °C, larvae were kept individually throughout the experiment at their designated temperature and also their behaviour was quantified at this temperature. Per female, larvae were randomly distributed across treatment combinations. Due to some mortality during the pre-exposure period, the number of larvae tested at each treatment combination varied between 28 and 50 (total of 303 larvae). Due to logistical constraints, a small number of larvae ($n = 13$) could only be tested for exploratory behaviour, and not for activity, food intake and boldness (Table 1).

Once larvae moulted into their final instar (when they were 161 ± 16 days old, mean ± SE, $n = 338$), they were randomly assigned to one of the pesticide treatments, consisting of an exposure to pulses of either pesticide or control solution for 15 days. The medium was renewed every three days (static renewal experiment). An ethanol-based chlorpyrifos stock solution with a concentration of 1 mg/ml was used to prepare a second, water-based stock solution of 10 µg/ml (renewed every week). Stock solutions were stored in the dark at 4 °C. The latter solution was used to prepare the final working concentration of 2 µg/l chlorpyrifos (nominal concentration), which was used for the pesticide pulses. This concentration was chosen because it modifies antipredator behaviour in coenagrionid damselfly larvae (Dinh Van et al., 2014b), and is within the range of chlorpyrifos concentrations reported in surface waters (Schulz, 2004). Pooled samples from ten different vials of the CPF treatment were analysed by the research laboratory Lovap NV (Geel, Belgium) using gas chromatography in combination with mass spectrometry (Thermo-Finnigan Trace DSQ). The initial chlorpyrifos concentration in the experimental cups was 1.24 µg/l. After three days (just before the renewal of the medium), the concentration was measured as 0.83 µg/l and 0.30 µg/l for the 20 °C and 24 °C temperature treatments, respectively. The control solution consisted of 2 µl/l ethanol to match the ethanol concentration in the chlorpyrifos treatment. We applied the pesticide (and control) solutions as six pulses during 15 days (three times per week). Throughout the exposure period, the larvae were fed daily the same amount of *Artemia* as during the pre-exposure period.

Larvae were weighed to the nearest 0.01 mg before and after the 15-day pesticide exposure period. Each larva was gently blotted dry with tissue paper before weighing to ensure that no water remained

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