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Adaptation of *Gammarus pulex* to agricultural insecticide contamination in streams

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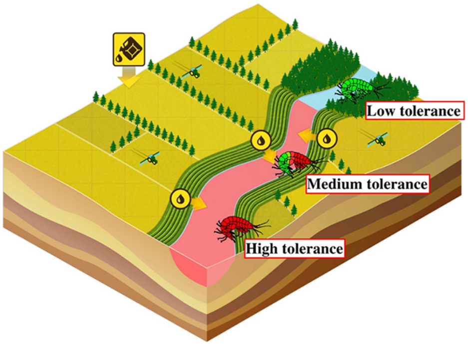
HIGHLIGHTS

- Non-target species in agricultural streams can acquire increased tolerance to pesticides.
- Tolerance and species composition were affected even at contamination below regulatory thresholds.
- The adaptation to pesticides significantly increased with local concentration.
- Non-contaminated refuge areas hindered local adaptation to pesticides.

GRAPHICAL ABSTRACT



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ABSTRACT

Exposure to pesticides affects non-target aquatic communities, with substantial consequences on ecosystem services. Adaptation of exposed populations may reduce the effects of pesticides. However, it is not known under which conditions adaptation occurs when only a low toxic pressure from pesticides is present. Here, we show that *Gammarus pulex*, a dominant macroinvertebrate species in many agricultural streams, acquires increased tolerance to pesticides when recolonization from non-contaminated refuge areas is low. Populations in the field that were exposed to pesticides at concentrations several orders of magnitude below considerable acute effects showed almost 3-fold higher tolerance to the neonicotinoid insecticide clothianidin (mean EC_{50} 218 μg L^{-1}) compared with non-exposed populations (mean EC_{50} 81 μg L^{-1}). This tolerance of exposed populations increased from 2- to 4-fold with increasing distance to the next refuge area (0 to 10 km). We conclude that the development of tolerance for non-target species may occur at very low concentrations, much below those affecting sensitive test organisms and also lower than those predicted to be safe by governmental risk assessment frameworks.

1. Introduction

Exposure to pesticides may affect the structure and function of freshwater non-target communities (Hunt et al., 2017; Liess and von der Ohe, 2005; Münze et al., 2017). Beketov et al. (2013) reported that pesticide pollution has significant effects on the species and family richness of

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macroinvertebrates in Australia and Europe, with losses of approximately one third of the taxonomic pools.

The repeated occurrence of toxic pressure may result in the weakening of exposed individuals (Russo et al., 2018), but also in the acquisition of increased tolerance towards pesticides by physiological acclimation or genetic adaptation (Becker and Liess, 2015; Becker and Liess, 2017; Klerks and Weis, 1987; Vigneron et al., 2015; Weston et al., 2013). Although the principles of adaptation to various pesticides are well-known, the roles of the magnitude of the toxic pressure and the prevailing environmental factors in the development of tolerance are still under debate. Developing a greater understanding of the relationship between environmental factors and tolerance to insecticides is of high relevance for the management of non-target species because the development of tolerance may have significant implications for ecology and conservation (Hua et al., 2013a). For example, pesticides can decrease genetic variation at the population level (Bijlsma and Loeschcke, 2012), which may reduce the ability to adapt to upcoming environmental changes (Bach and Dahllöf, 2012; McMillan et al., 2006). However, resistant non-target populations can minimize the effects of pesticide-induced trophic cascades (Bendis and Relyea, 2016).

In addition to the local toxic pressure, non-contaminated refuge area is an important factor that drives the development of pesticide resistance. Recolonization of sensitive individuals from refuges can partially compensate the selection for pesticide resistance in agricultural fields (Gassmann et al., 2009). In the same way, the recolonization of sensitive species from upstream refuges can partially compensate the effects of pesticides on the macroinvertebrate community at downstream sections (Bunzel et al., 2014; Orlinskiy et al., 2015; Von der Ohe and Goedkoop, 2013). However, the impact of refuges on the resistance development in non-target species is still unclear, in spite of the relevance for the risk assessment of pesticides and the planning of mitigation measures.

The aim of this investigation is to reveal the extent to which low pesticide contamination induces adaptation in aquatic non-target species. Additionally, we aim to assess the environmental parameters that govern the development of increased pesticide tolerance. For this purpose, we selected *Gammarus pulex* (Linnaeus, 1758), a benthic macroinvertebrate as test organism because of its ecological relevance in aquatic ecosystems. *Gammarus pulex* is one of the most common freshwater macroinvertebrates and widely distributed in Europe. It plays a central role in the degradation of organic matter (Foucreau et al., 2013; Maltby et al., 2002; Mora Gómez, 2014) and constitutes an imperative element in the food web (Macneil et al., 1999). Numerous investigations in the laboratory have reported detrimental effects of pesticides on reproduction, feeding behavior and survival in *Gammarus pulex* and related crustaceans that results in reduced leaf litter degradation (Agatz et al., 2014; Baudy et al., 2017; Cold and Forbes, 2004; Nyman et al., 2013). *Gammarus pulex* is sensitive to a wide range of chemicals and has been frequently used for risk assessment (Adam et al., 2009; Agatz et al., 2014; Maltby et al., 2002; Vigneron et al., 2015), However, field studies suggested that the species is able to recover well from pesticide exposure through reproduction and recolonization, and therefore considered it not at high risk (Liess and von der Ohe, 2005; Rasmussen et al., 2012; Schäfer et al., 2012).

2. Materials and methods

2.1. Location and physicochemical description of investigated streams

In total, 15 sites were investigated in 2015 and 2016 within central Germany that cover a wide range from uncontaminated to highly contaminated streams (Fig. S1). In order to exclude the contaminants other than pesticides, it was ensured that the investigated sites had no wastewater treatment plants, industrial facilities, or mining drainage upstream. Sampling sites were characterized by soft- and hard-bottom substrates in different proportions. Major crops in the study area were wheat, rapeseed, sugar beets, corn and barley. Of the 15 sites, six were located in less contaminated forested areas and used as control sites. Populations from forested streams are generally not contaminated in Germany with the exception of rare accidents (Zwick, 1992). In contrast, streams with an agricultural catchment were not protected from pesticide contamination and most likely to experience higher pesticide contents.

During the sampling, parameters such as the water level, electrical conductivity (EC), pH, temperature and dissolved oxygen level (DO) were measured. Additionally, undisturbed forested stream sections that may serve as refuge areas were identified. The distance to the closest undisturbed forested stream section was measured using Google Maps. We considered both upstream and downstream refuge sections because *Gammarus pulex* can migrate in both directions.

2.2. Characterization of pesticide contamination

2.2.1. Sampling

Water samples were collected from all the selected sites using event-driven water samplers (EDSs) (Liess and von der Ohe, 2005) in summer 2016, during the period of pesticide application. For this purpose, two glass bottles were installed at heights of 5 and 15 cm from the level of the stream water to collect rainfall-induced short-term maximum pesticide contamination. The bottles were collected within 24 h after rainfall events and transported to the laboratory at 4 °C. After settlement of the particles, 1 mL aliquots from the top 1 cm of the bottle were extracted and placed into 2 mL autosampler vials and then stored at −20 °C until analysis.

2.2.2. Chemicals and reagents

For the liquid chromatography–high-resolution mass spectrometry (LC-HRMS) analyses, we used methanol, water and formic acid of LC-MS grade (Chromasolv, Sigma-Aldrich, Germany). Stock solutions of the target analytes were prepared in methanol at 1 mg mL^{-1} and stored in amber glass vials (20 mL) at −20 °C in the dark. Mixed solutions of 10 μg mL^{-1} were prepared in methanol and used for identification and calibration.

2.2.3. LC-HRMS target screening

The water sample aliquots (1 mL in 2 mL autosampler vials) received 25 μL of an internal standard solution (40 ng mL^{-1} of isotope-labeled compounds in methanol), 25 μL of methanol and 10 μL of a 2 M NH_4-formate buffer (pH 3.5).

For the analysis, a Thermo Ultimate 3000 LC system (consisting of a ternary pump, auto sampler and column oven) was coupled to a quadrupole-orbitrap instrument (Thermo QExactive Plus) via a heated electrospray ionization (ESI) source. LC separation was performed on a Kinetex C18 EVO column (50 × 2.1 mm, 2.6 μm particle size) using a gradient elution with 0.1% of formic acid (eluent A) and methanol containing 0.1% formic acid (eluent B) at a flow rate of 300 μL/min. After 1 min of 5% B, the fraction of B was linearly increased to 100% within 12 min, and 100% B was maintained for 11 min. The eluent flow was diverted to waste, and the column was rinsed for 2 min using a mixture of isopropanol + acetone (50:50)/eluent B/eluent A (85%/10%/5%) to remove hydrophobic matrix constituents from the column. Finally, the column was re-equilibrated to initial conditions for 5.7 min. The injection volume was 100 μL, and the column was operated at 40 °C. The heated ESI source and the transfer capillary were both operated at 300 °C, the spray voltage was 3.8 kV (pos. mode) or 3.5 kV (neg. mode), the sheath gas flow rate was 45 a.u., and the auxiliary gas flow rate was 1 a.u. Separate runs were conducted in positive and negative ion mode by combining a full scan experiment (100–1000 m/z) at a nominal resolving power of 70,000 (referenced to m/z 200) and data-independent MS/MS experiments at a nominal resolving power of 35,000. For the latter, we acquired the data using broad isolation windows of approximately 50 (i.e., m/z ranges 97–147, 144–194, 191–241, 238–288, 285–335, 332–382, 379–429, 426–476) and 280 (i.e., m/z ranges 460–740, 730–1010).