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Sequential exposure to low levels of pesticides and temperature stress increase toxicological sensitivity of crustaceans

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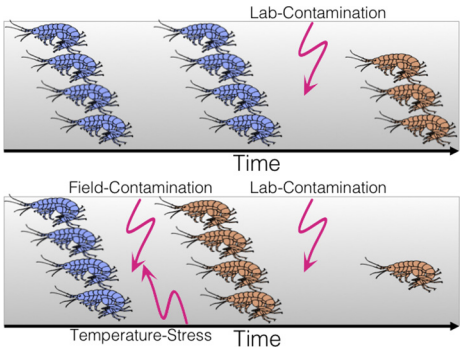
HIGHLIGHTS

- Agricultural streams are subjected to sequential pesticide contaminations.
- Environmental stressors interact with pesticides in the field.
- Crustaceans from agricultural streams showed increased toxicological sensitivity.
- A synergistic interaction between pesticide and temperature stress was revealed.
- A realistic risk assessment needs to account for this pesticide-stress interaction.

GRAPHICAL ABSTRACT



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ABSTRACT

Frequent pesticide-related impacts on ecosystems at concentrations considered environmentally safe indicate that the current risk assessment framework for registration of pesticides is not protective enough. Causes may include difficulties in assessing the effects of sequential pesticide pulses and their interaction with environmental stressors. By contrast to such realistic scenarios, risk assessment for registration of pesticides is typically based on tests of a single exposure period under benign laboratory conditions. Here, we investigated the toxicological sensitivity of *Gammarus pulex*, an ecologically relevant crustacean, from uncontaminated control streams and pesticide-contaminated agricultural streams by exposing them to pesticide contamination in the laboratory. Individuals from contaminated streams were 2.7-fold more sensitive to pesticide exposure than individuals from the reference streams. We revealed that this increase in sensitivity was the result of a synergistic interaction of sequential pesticide exposure and temperature stress. Such multiple stressor scenarios are typical for agricultural streams. We conclude that the interactive effects of sequential toxicant exposure and additional environmental stressors need to be considered in a realistic risk assessment framework.

1. Introduction

Risk assessment for the registration of pesticides has been established to protect non-target communities. To address uncertainties related to the projection of toxicity assessments from benign laboratory conditions towards the field conditions and to predict the regulatory acceptable concentration (RAC) (Office of pesticide programs U.S.

Abbreviations: CS, contaminated sites; US, uncontaminated sites.

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environmental protection agency, 1999), an assessment factor of 100 below the acute LC_{50} (concentration that is lethal to 50% of the test organisms) has been established. However, the impacts of pesticides on the structure (Liess and Von Der Ohe, 2005; Schäfer et al., 2012) and the biodiversity (Beketov et al., 2013) of agricultural streams have been observed frequently. Non-compliance with regulations during agricultural practices may contribute to this problem; however, frequent occurrence of environmental impacts of pesticides indicates that the current framework of risk assessment omits relevant processes that determine ecologically effective concentrations. The European Food Safety Authority (EFSA) lists several sources of uncertainty in the projection from test systems to the field (European Food Safety Authority (EFSA), Parma, Italy, 2013), including sequential exposure to a mixture of pesticides within one generation (Ashauer et al., 2007), combined effects of pesticides and environmental stressors (Liess et al., 2016) and culminating effects induced by sequential contamination over several generations (Liess et al., 2013).

Single short-term pulses of pesticides that are typical in agricultural streams (Liess et al., 1999; Handy, 1994) are known to cause delayed adverse effects on aquatic invertebrates (Abel, 1980) and fish (Floyd et al., 2008). For example, Liess (2002) showed that caddisflies exposed to fenvalerate for 1 h at 1/1000 of the acute LC_{50} suffered increased mortality 8 months after this brief exposure. Similar delayed effects of short-term exposure were identified for various invertebrate species exposed to the insecticides esfenvalerate (Beketov and Liess, 2005), thiacloprid (Liess et al., 2013; Beketov and Liess, 2008), imidacloprid (Nyman et al., 2013; Agatz et al., 2014) and endosulfan (Barry and Logan, 1998). Apparently, short-term exposure to a toxicant may result in long-term weakening of individuals that can cause the observed delayed effects. Furthermore, recent investigations showed that sequential exposure patterns may progressively increase the sensitivity of soil (Jordaan et al., 2012; Reinecke and Reinecke, 2005) and aquatic (Ashauer et al., 2017; Ashauer et al., 2015) organisms within one generation. Toxicokinetic and toxicodynamic (TK/TD) models, such as the GUTS (General Unified Threshold model for Survival) approach (Ashauer et al., 2016) have been suggested to predict the effects of sequential pulse exposure; it accounts for variable toxicant exposure over time, allowing the prediction of survival after different exposure patterns and time-scales. However, they are not validated in the field context where environmental stressors are present. For a realistic assessment of pesticide effects in the field, this additional stress needs to be included. Recently, a meta-analysis has shown that combined stressors increased toxicological sensitivity of organisms by more than one order of magnitude (Liess et al., 2016).

Accordingly, in the present study, we investigated whether the sensitivity of *Gammarus pulex* in agricultural streams was affected by sequential exposure to pesticides in combination with additional environmental stressors.

2. Materials and methods

2.1. Study design

Gammarus pulex (Linnaeus, 1758) was selected as the test organism because of its ecological relevance in many stream ecosystems. It plays a pivotal role in the degradation of allochthonous leaf litter (Dangles et al., 2004), a crucial ecosystem function that can be disturbed by pesticide contamination (Brosed et al., 2016). *G. pulex* individuals were sampled from four uncontaminated stream sections and from four sites contaminated with agricultural pesticides, and they were subsequently exposed to the pyrethroid insecticide esfenvalerate in the laboratory. Sampling of test organisms was scheduled according to the expected regime of insecticide exposure in the field, which was estimated based on the 2015 governmental recommendations for pesticide application in Saxony, Saxony-Anhalt and Lower Saxony (Germany) provided by the Saxony-Anhalt State Institute for Agriculture, Forestry and Horticulture (LLFG), Bernburg (Fig. S1). At each site, crustaceans were sampled during the following three time periods:

(i) Autumn (October 2015): 3–4 months after maximum pesticide application (Liess et al., 1999; Huber et al., 2000), corresponding to "no/low pesticide exposure" in the field (Fig. S1);
(ii) Spring (March–April 2015): at the beginning of pesticide application (Liess et al., 1999; Huber et al., 2000), corresponding to "low pesticide exposure" in the field (Fig. S1); and
(iii) Summer (June 2015): during maximum pesticide application (Liess et al., 1999; Huber et al., 2000), corresponding to "highest pesticide exposure" in the field for the three sampling periods (Fig. S1).

Notably, *G. pulex* produces two or three overlapping generations per year (Welton, 1979a). Reproduction is particularly low in winter. Therefore, individuals sampled in early spring generally belonged to the same generation as those that found in the previous autumn. By contrast, reproduction strongly increases in early summer causing high generation turnover from spring to autumn. Accordingly, in our study, we sampled different generations of *G. pulex* according to the timing of field campaigns.

2.2. Selection of the study sites

Selected streams were located in Saxony and Saxony-Anhalt, Germany (Fig. S2) and they were characterized by the following parameters: average width of 2 m, average depth of 0.3 m, available hard structure (i.e., stones and wood) in the sampling area, buffer strips on both banks and an uncontaminated refuge area within a range of 5–15 km up- or down-stream from the sampling point. Additionally, we selected sites with no wastewater treatment plants present within at least 3.0 km upstream to exclude exposure to contaminants other than pesticides. Water conductivity, temperature and pH were measured at each sampling date. Due to technical issues (i.e., low water levels), we could not use the complete data set of 24 observations (8 observations for each of the 3 field campaigns) for all analyses (Table S4). In the following sections, the data set used for each analysis is specified.

2.3. Assessment of pesticide exposure and categorization of sampling sites

It is highly demanding to accurately assess the overall exposure of aquatic invertebrates to pesticides in the field. Discharge of pesticides generally occurs in several peaks (Liess et al., 1999; Handy, 1994) driven by rainfall events with run-off. Such sequential exposure to various pesticides exerts complex mixture effects. Additionally, bioavailability and effects of toxicants depend on various parameters including suspension load (Schulz and Liess, 2001), temperature (Harwood et al., 2009) and behavior of individuals (Rasmussen et al., 2013). Because of these considerations, we quantified the magnitude of toxic pressure with a biological measure, the indicator $SPEAR_{pesticides}$ (Liess and Von Der Ohe, 2005). This indicator system analyzes the community composition of macroinvertebrates at a given site to estimate toxic pressure on invertebrates. It provides the advantage of assessing the overall biological effects of pesticides, including the bioavailability and the combined effects of sequential exposure and mixtures of pesticides (Liess and Von Der Ohe, 2005; Schäfer et al., 2012; Münze et al., 2017).

Macroinvertebrate communities were sampled in early summer during maximum pesticide exposure (Liess et al., 1999), the time period for which the SPEAR index has been validated and best indicates pesticide exposure (Liess and Von Der Ohe, 2005; Schäfer et al., 2012; Orlinskiy et al., 2015a; Münze et al., 2015). At each stream, ten subsamples were collected across different habitats to obtain a representative sample of the macroinvertebrate community (according to the protocol for SPEAR sampling, http://www.systemecology.eu/spearcalc/index.en.