Title

Insecticides in agricultural streams exert pressure for adaptation but impair performance in *Gammarus pulex* at regulatory acceptable concentrations

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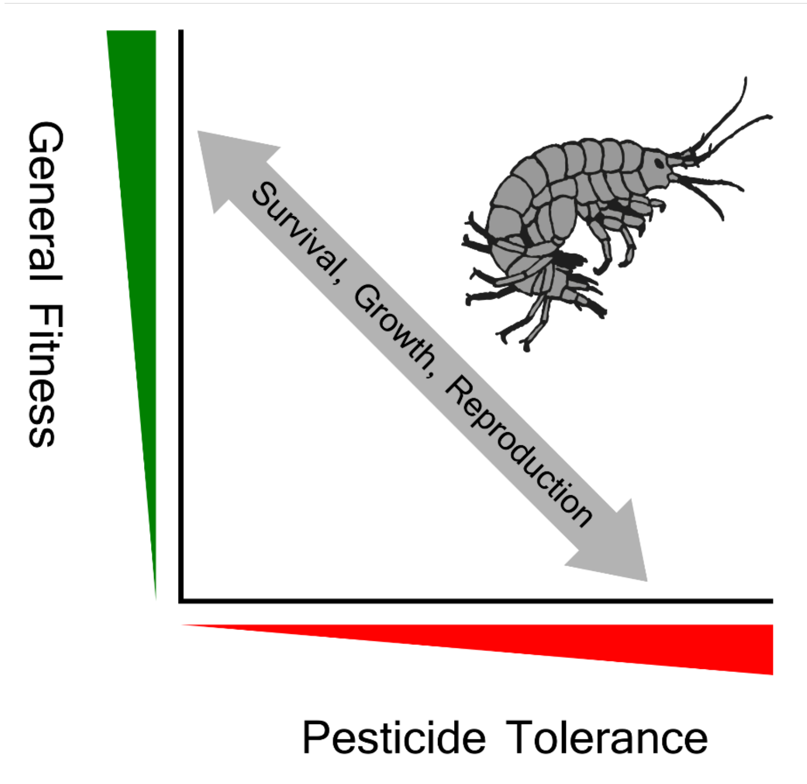

Graphical abstract

Abstract

Pesticide exposure in agricultural streams requires non-target species to adapt. However, pesticides may reduce performance in between exposure events due to long-term effects and physiological fitness costs of adaptation. Here, we investigated the long-term consequences of pesticide exposure to low concentrations in the widespread crustacean *Gammarus pulex*.

We collected populations from six German streams covering no to moderate agricultural pesticide exposure. Peak concentrations ranged up to 1/400 of their acute median lethal concentration (Toxic Unit = -2.6), resulting in significant changes in the macroinvertebrate community composition ($SPEAR_{pesticides}$ = of 0.2). Acute toxicity tests revealed up to a 3-fold increased tolerance towards the most frequently found insecticide clothianidin compared to individuals collected from non-contaminated streams. However, populations showing increased insecticide tolerance were characterized by reduced survival, per capita growth and mating adults when cultured under pesticide-free conditions in the laboratory for three months.

We conclude that pesticide pollution triggers adaptation both at the species and the community level even at concentrations considered to be safe according to the European pesticide legislation. In *G. pulex*, adaptation is associated with impaired performance which potentially affects ecosystem functions such as leaf litter degradation. These long-term impairments need to be considered in deriving safe concentrations.

Key words*: Gammarus pulex*, Fitness costs, Pesticide exposure, Adaptation, Neonicotinoids, Ecotoxicology

Introduction

Macroinvertebrates in agricultural streams are exposed to pesticides, especially during the surface runoff following rainfall events (Kreuger, 1998; Liess and Schulz, 1999). This pesticide exposure may extend from a few minutes to several hours, depending on the intensity of rainfall, pesticide properties and the characteristics of the water body. Pesticide exposure poses deleterious effects on the structure and functions of macroinvertebrates in agricultural streams (Hunt et al., 2017; Liess and von der Ohe, 2005; Münze et al., 2017; Shahid et al., 2018). Furthermore, Beketov et al. (2013) reported significant effects of pesticide contamination on the species and family richness of macroinvertebrates. Significant change in the macroinvertebrate communitiy composition has been reported even at 3 to 4 orders of magnitude below the acute median lethal concentration (LC_{50}) of laboratory standard test species (Liess and von der Ohe, 2005; Schäfer et al., 2012). Reasons for these low environmental effect concentrations include an increased sensitivity of individuals to pesticides under multiple stress conditions (Liess et al., 2016; Liess et al., 2019) and the culmination of effects from sequential exposure (Liess et al., 2013). Accordingly, the exposure to pesticides may exert a considerable pressure for adaptation, which results in increased pesticide tolerance in exposed species (Becker and Liess, 2015; Delnat et al., 2019; Sparks and Nauen, 2015; Vigneron et al., 2015; Weston et al., 2013). Recently, Becker and Liess (2017) and Shahid et al. (2018) reported 3- to 4-fold increased pesticide tolerance in *Gammarus pulex* collected from agricultural streams.

However, adaptation to pesticides is often associated with considerable fitness costs under non-toxic conditions (Becker and Liess, 2015; Delnat et al., 2019; Medina et al., 2007; Meyer and Di Giulio, 2003). Fitness costs of increased pesticide tolerance (resistance) may reduce ability for population recovery in between events of pesticide exposure. Bach and Dahllof (2012) revealed cost of resistance to high pesticide concentrations (causing acute mortality in non-adapted populations) in marine amphipod collected from contaminated fjord. Similarly, (Heim et al., 2018) reported lower reproductive capacity and lower upper thermal tolerance in pyrethroid-resistant *Hyalella azteca* individuals compared to non-resistant individuals. Obviously, coping with the toxicity of high insecticide concentrations is costly, and requires energy and resource allocation for adaptation and survival. However, fitness costs of resistance to lower pesticide concentrations that typically cause no acute mortality but sub-lethal effects ($TU \leq -3$) have never been investigated. Such concentrations have been observed regularly in the field (Knillmann et al., 2018; Liess and von der Ohe, 2005) and are generally considered to be safe according to the European pesticide legislation (Products and Residues, 2013). Unraveling these effects in real field conditions may contribute to our understanding of why pesticides affect the macroinvertebrate community composition in the field at concentrations much lower than those predicted to be safe based on the sensitivity tests in the laboratory (Liess et al., 2013).

This study aimed at investigating the effects of pesticide exposure on the tolerance and fitness of *Gammarus pulex* which is one of the most common freshwater macroinvertebrate species in central European streams with high ecological relevance for leaf litter degradation (Schäfer et al., 2012). We were particularly interested in potential effects of increased pesticide tolerance on key life-history traits. For this purpose, populations from agricultural and reference streams with different tolerances to pesticides were cultured under uncontaminated conditions and the variable: survival, per capita growth and reproduction were monitored for three months.

2. Materials and Methods

2.1. Study design

We collected *Gammarus pulex* from six sites that covered a range of non-contaminated to moderately contaminated streams in central Germany. The test organisms were sampled from April to June 2018 during the period of peak pesticide application. Additionally, macroinvertebrates were sampled to quantify effects of pesticide exposure on the community composition using the $SPEAR_{pesticides}$ bioindicator. Pesticide exposure during the study period was measured from water samples collected during run-off events after heavy rainfalls. The organisms were acclimatized to test conditions for seven days and subsequently exposed to six different concentrations of the neonicotinoid insecticide clothianidin. Additionally, we cultured each population in a climate chamber under standardized pesticide free conditions for three months. Long-term endpoints such as survival, per capita growth and reproduction of cultured organisms were recorded.

2.2. Study sites

Three sites were located in less contaminated forested area and selected as reference sites, whereas streams close to agricultural fields were not protected and showed considerably higher pesticide exposure (Table 1). None of these sites were influenced by other sources of contaminants such as wastewater treatment plants (WWTPs), industrial effluents or mining drainage. During the sampling, different physico-chemical parameters such as electrical conductivity (EC), temperature, pH, and dissolved oxygen (DO) were also measured showing that these environmental parameters were well in the range of favourable conditions for *G. pulex* (McCahon and Pascoe, 1988b)(Table 1).

Table 1. Pesticide exposure and physico-chemical properties of the study sites.

Site	Coordinates	EC (µS)	Temperature	pH	DO	SPEAR	TU_{max}	Most toxic compound	Compound class
Ref-1	50.59251 10.64666	728	14	8.5	-	0.91	-5.34	2_4_Dichlorophenoxy -acetic acid	Phenoxy herbicides

Ref-2	52.1656 10.83203	756	8.9	7.56	8.94	1.00	-5.83	Fluroxypyr	Pyridine herbicides
Ref-3	51.33528 12.97136	534	15.8	7.63	-	0.69	-3.69	2_4_Dichlorophenoxy-acetic acid	Phenoxy herbicides
Agri-1	51.28995 12.15237	2,060	15.4	8.08	11.5	0.09	-2.65	Thiacloprid	Neonicotinoids
Agri-2	51.46098 11.47198	1,033	15.7	8.5	11.85	0.34	-3.63	Thiacloprid, clothianidin	Neonicotinoids
Agri-3	52.27735 10.75138	1,839	15.9	8.27	14.7	0.37	-2.79	Thiacloprid, clothianidin	Neonicotinoids

2.3. Sampling of macroinvertebrates and test organisms

Macroinvertebrates were collected from 20 subsamples along a 50 m stream section, following the German nationalguidelines for the biological monitoring of streams (Gellert et al., 2014). In short, we collected organisms using a kick-sampler (25 x 25 cm) with a mesh size of 500µm, sorted specimens in a white tray and preserved them in 70 % EtOH for subsequent taxonomic identification in the laboratory. Individuals of *Gammarus pulex* were collected using a spoon or pipette and transported to the laboratory under constant aeration in a cooling box. Every week, a highly polluted and a lowly polluted population were sampled and tested to avoid a potential bias that may result from temporal variation in pesticide tolerance.

2.4.Quantification of pesticide exposure

Water samples from all sites were collected under the KgM project using two different techniques. The number of samples from each site varied from 1 to 6, depending on rainfall events. Briefly, computer triggered event samplers (Liess et al., 1999) and water level triggered event samplers (Liess and von der Ohe, 2005) were installed at each site. Water samples were collected within 24h following rainfall events, kept in a cool box at 4 °C and transported to the laboratory. Afterwards, 1 mL aliquots were transferred into 2 mL autosampler vials and stored at −20 °C for analysis. A total of 108 chemicals, including insecticides, fungicides and herbicides were analysed in water samples collected from selected streams. Targeted substances were analyzed using LC-

HRMS (Ultimate 3000 LC system coupled to a QExactive Plus MS equipped with a heated electrospray ionisation (ESI) source, all from Thermo Scientific).

To quantify toxicity, mean concentrations of pesticides detected in streams were converted into toxic units (TU) by relating the measured concentration of a pesticide to its median lethal concentration for a sensitive referece species (Sprague, 1970). Existing field studies in small freshwater streams show that pesticide effects on macroinvertebrate communities are mainly related to the maximum toxic unit (TU_{max}) exerted by the single most toxic compound. The summed up toxic units of all compounds (TU_{sum}, based on the assumption of additivity) does typically not increase the explained variance of biological effects significantly (Liess and von der Ohe, 2005; Orlinskiy et al., 2015; Schäfer et al., 2012). Therefore, we calculated the maximum toxic unit (TU_{max}) following the equation given below (Liess and von der Ohe, 2005; Tomlin, 2009) and used for further calculations.

$$TU_{max} = \mathrm{Max}_{i=1}^{n}\left[\log\left(\frac{Ci}{LC_{50i}}\right)\right] \qquad (1)$$

where TUmax is the highest value of *n* pesticides at each sampling site, C_i is the concentration (μg L^{-1}) of pesticide *i*, and LC_{50i} is the median lethal concentration (48 h, μg L^{-1}) of that pesticide for the reference organism. Here, we used LC_{50} values for *Daphnia magna*, *Chironomus riparius*, *Chironomus tentans*, *Hyalella azteca* obtained from the Ecotoxicology Database System (USEPA) and Pesticide Properties Database (PPDB), and the most sensitive species was selected as reference.

2.5. Effects of pesticide exposure on the macroinvertebrate community

The $SPEAR_{pesticides}$ (SPEcies At Risk) bioindicator quantifies the toxic pressure of pesticides based on the proportion of macroinvertebrates classified as highly vulnerable to pesticides ("SPEcies At Risk") to the total number of macroinvertebrates (Liess and von der Ohe, 2005). We calcuated $SPEAR_{pesticides}$ values using the software INDICATE (V 2019.11) that contains latest version of SPEAR index recently upgraded according to (Knillmann et al., 2018). A low proportion of vulnerable taxa (low SPEAR value) indicates high effects of pesticides and thus high pesticide exposure in a stream.

2.6. Acute toxicity tests for pesticide tolerance

The acute sensitivity of *Gammarus pulex* to neonicotinoid insecticide clothianidin was tested based on the OECD guidelines for the testing of chemicals (OECD 2004) and the guidelines for rapid tests for community-level risk assessment (Kefford, 2013). We selected the neonicotinoid insecticide clothianidin as test substance because it represents one of the most commonly applied classes of insectides in agriculture and has been frequently detected in surface waters (Knillmann et al., 2018).Test organisms were acclimatized to the test conditions in the laboratory for seven days before exposure. A 40 mg/L stock solution of clothianidin was prepared in distilled water using DANTOP (500g/kg, Spiess-Urania Chemical GmbH, Germany) and stirred overnight on magnetic stirrer. Required test concentrations i.e., 0, 5, 19, 72.2, 274.4, and 1042.6 μg/L were prepared by diluting the stock solution in ADAM (Artificial Daphnia medium) (Klüttgen et al., 1994). Random samples of the test solutions were analyzed for control; the average and maximum measured concentration of each nominal concentration ranged within acceptable boundaries (± 10%). Briefly, 16 individuals from each population were exposed to each test concentration of clothianidin for a period of 96 h to determine the effect of the toxicant. After every 24 h, the immobility and the mortality of the individuals were recorded as end points. Individuals were considered to be immobile if they did not move their bodies within 30 s of undisturbed observation or after probing with a rod. The fanning of gills and antenna was not counted as body movement.

2.7. Culture of Gammarus pulex

The long-term effects of field exposure were analysed by culturing *Gammarus pulex* under standardized pesticide-free conditions in the laboratory. In general, maintenance and culturing of organisms followed the descriptions given by (McCahon and Pascoe, 1988a, b) with modifications as follows: Approximately 100 individuals from each population including medium-sized gammarids were cultured in 5L glass tanks with a 3L aerated artificial stream water (ADaM) (Klüttgen et al., 1994) was used as culture medium. Organisms were fed with Alder leaves pre-conditioned in stream water for at least two to three weeks before use. Pre-conditioned stones of different size were added to increase the water quality of the culture medium and to provide juveniles with the opportunity to hide from omnivorous adults. Continuous aeration was provided using potable air pumps to avoid stress during experiments and cultures. Aeration and food availability was checked regularly. To maintain the quality of the medium, 500 mL of old culture medium was replaced by fresh medium after 14 days. Additionally, 1,500 mL of old medium was replaced with fresh medium after 30 days and dead organisms were removed. The culture was maintained at 16°C, with 60% humidity and artificial light (12 h light:12h dark) in a climate chamber. To analyze the long-term effects of local pesticide contamination in streams on respective populations, the survival, per capita growth and the proportion of mating adults were monitored every four weeks for three months.

2.8. Data analysis

All statistical analyses were carried out using R Studio for Windows (V 1.2.1) and R for Windows (V 3.5.1). For each acute sensitivity test and each observation time, the concentration that affected

75% of the exposed population (EC_{75}) was calculated using 5-parameter non-linear regression available with the package drc (V 3.0-1) (Ritz et al., 2015). We used a binomial error distribution in the models and set the lower and upper boundary to 0 and 1, respectively. EC_{75} was used instead of EC_{50} to quantify pesticide tolerance because we observed that the increase in tolerance with pesticide pollution was more pronounced at higher effective concentrations. This is in accordance with the finding of Becker et al. (in preparation). To make use of all data, we then performed linear regression of all log-transformed EC_{75} values from the same test vs. the log-transformed observation time and interpolated the EC_{75} after the mean test duration (60h). This 60h EC_{75} was used as a measure of pesticide tolerance for further analyses. The long-term end points for the cultured populations after three months were derived from the introduced adults and their (pre-adult) offspring which could be differentiated based on size. Survival was calculated by simply dividing the number of adult individuals by the initial number of individuals at the start of the culture. For per capita growth, the total number of offspring was divided by the initial number of individuals. The proportion of mating adults ("reproduction") was calculated as 2 x the number of couples , divided by the overall number of adults in the culture.

Simple linear regression was applied to analyze the correlation of pesticide pollution in the field (TU_{max}) with pesticide tolerance (log-transformed EC_{75}) and with the $SPEAR_{pesticides}$ bioindicator. The effects of of pesticide pollution and of pesticide tolerance on survival and reproduction after three months culture in the laboratory was analyzed using a binomial generalized linear model with a logit link function. The effects of pesticide pollution and of pesticide tolerance on the (log-transformed) per capita growth were analyzed using simple linear regression.

The assumptions of homoscedasticity and of normally distributed residuals were confirmed by visual inspection, plotting residuals vs. fitted values residuals vs. leverage, and Q-Q plots.

Results

3.1. Pesticide contamination and the effects on community structure

Among investigated sites, the maximum toxic unit (TU_{max}) ranged from −5.8 to −2.6. In agricultural streams, most toxic compounds were neonicotinoids (clothianidin; *n* = 1, TU_{max} = −3.6 and thiacloprid; *n* = 2, mean TU_{max} = −2.7). In contrast, the forested streams that served as controls were only slightly contaminated. The maximum toxic unit ranged from −5.8 to −3.7 which is considered to be of lower ecotoxicological relevance. In all forested sites, a herbicide (2, 4−Dichlorophenoxyacetic acid) was responsible for maximum toxicity (n=3, mean TU_{max}= −4.96).

The toxic pressure exerted by pesticides on the macroinvertebrate community structure was quantified using the $SPEAR_{pesticides}$ indicator. The change in $SPEAR_{pesticides}$ was strongly correlated to the TU_{max} of respective streams (linear regression, F = 23.9, residual df = 4, adjusted R^2 = 0.82, *p*-value = 0.008; Figure 1). Agricultural streams characterized by higher TU_{max} showed significantly lower $SPEAR_{pesticides}$ values (0.08 − 0.37) in comparison to the forested reference streams with lower pesticide contamination (0.7 − 1.0) (Welch Two Sample t-test, *p-value* < 0.01). These effects were observed even at concentrations in the range of 1/1000 to 1/100 of the acute LC_{50} of the most sensitive standard test organism that is generally considered to be safe by governmental risk assessment frameworks (European Commission, 2011).

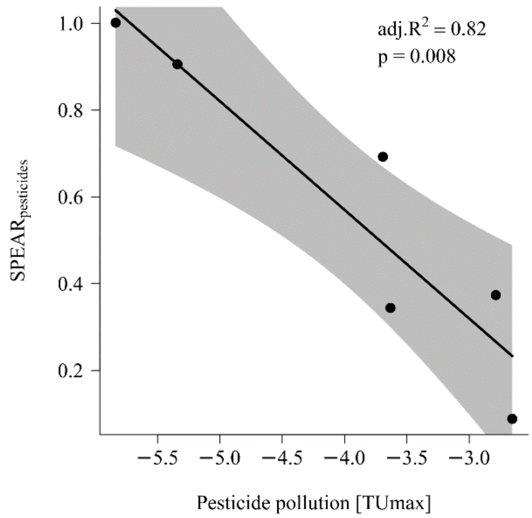

Figure 1. Correlation between the macro-invertebrate community structure indicated as $SPEAR_{pesticide}$ and the stream contamination expressed as maximum toxic unit (TU_{max}). The grey area corresponds to 95% confidence interval.

3.2. Increased tolerance to clothianidin in Gammarus pulex

The median effective concentration of the insecticide clothianidin after 96 h exposure (96h LC_{50}) was on average 33.28 µg/L across all populations. This was comparable to the 96h LC_{50} of clothianidin reported for the most sensitive standard test species used for the calculation of TU_{max} (*Chironomus riparius*, 29 µg/L). Therefore, we consider pesticide effects in *Gammarus pulex* as representative also for other sensitive macroinvertebrates.

We observed pesticide adaptation already at low contamination in the range of TU_{max} = -3. *G. pulex* from agricultural streams was 2−fold more tolerant (mean effective concentration that immobilized 75 % of test individuals after 60h, EC_{75} = 158 µg/L) to clothianidin compared to populations from reference streams (mean EC_{75} = 71 µg/L; t = -3.03, residual df = 4, p-value =

0.038). The tolerance to clothianidin was significantly correlated with the stream contamination expressed as TU_{max} (linear regression; F = 8.6, residual df = 4, adjusted R^2 = 0.61, p-value = 0.042, Figure 2). Further, clothianidin tolerance also showed a correlation with the macroinvertebrate community structure expressed as $SPEAR_{pesticides}$ (linear regression; adjusted F = 5.67, residual df = 4, R^2 = 0.48, p-value = 0.075) that also provides information about the local pesticide contamination.

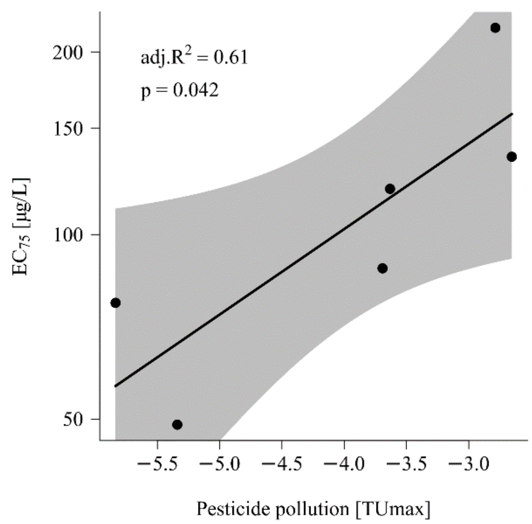

Figure 2. Correlation between pesticide tolerance in *Gammarus pulex* (quantified as EC_{75} of the insecticide clothianidin after 60h constant exposure, log-transformed) and the level of pesticide pollution in the field (quantified as maximum toxic units, TU_{max}). Means ± 95 % confidence intervals are shown.

3.3. Consequences of increased tolerance in Gammarus pulex

We observed that the increased tolerance to clothianidin (60h EC_{75}) had consequences on the general fitness and long-term viability of *G. pulex*. Populations from agricultural streams with increased clothianidin tolerance showed reduced survival in culture after three months, as compared to populations from less contaminated reference streams (generalized linear regression; *Chi²* = 7.87, residual df = 4, Zavoina's R^2 = 0.23, *p*-value = 0.005, Figure 3a). Similarly, the per capita growth of gammarid populations significantly decreased with increasing clothianidin tolerance expressed as EC_{75} (linear regression; F = 13.72, residual df = 4, adjusted R^2 = 0.72, *p*-value = 0.021, Figure 3b). Furthermore, increased pesticide tolerance (EC_{75}) was significantly associated with a reduced proportion of mating adults in the populations (generalized linear regression; *Chi²* = 6.12, residual df = 4, Zavoina's Pseudo R^2 = 0.32, p-value = 0.013, Figure 3c).

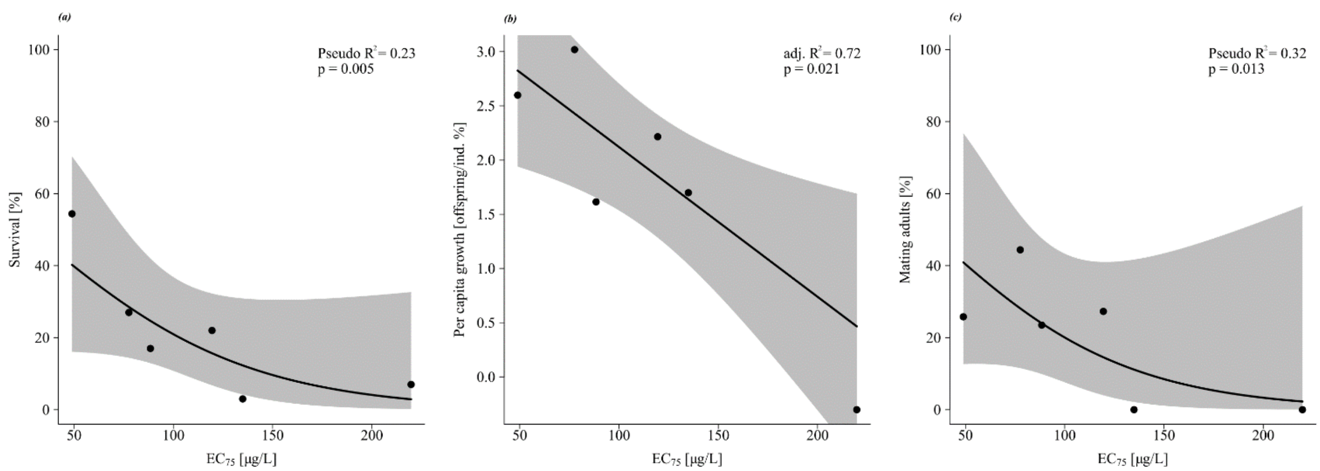

Figure 3. Effect of pesticide tolerance (60h EC_{75} of the insecticide clothianidin) on (a) survival, (b) per capita growth, and (c) reproduction (proportion of mating adults) of *Gammarus pulex* populations. The grey area corresponds to 95% confidence interval.

Performance in the cultured populations correlated also with the pesticide pollution observed in the field: Increasing pesticide contamination (TU_{max}) was associated with reduced survial (generalized linear regression, *Chi²* = 2.33, residual df = 4, Zavoina's Pseudo R^2 = 0.084, *p*-value = 0.12), per capita growth (linear regression, F = 5.11, R^2 = 0.45, residual df = 4, *p*-value = 0.086) and a lower proportion of mating adults (generalized linear regression, *Chi²* = 2.14, residual df = 4, Zavoina's Pseudo R^2 = 0.11, *p*-value = 0.14, Table S1).

4. Discussion

4.1. Quantification of pesticide effects on macroinvetebrate community structure

In the present study, the pesticide contamination (TU_{max}) of agricultural streams ranged from −5.8 to −2.6. According to the most conservative first tier evaluation of European regulations for agricultural pesticides (Products and Residues, 2013), generally no effects should occur at concentrations 100 times below the LC_{50} of reference organisms (TU_{max} = -2). However, we found strong effects of pesticide pollution on the macroinvertebrate community composition at considerably lower TU_{max} levels. These ecological effects (quantified with the SPEAR index) are consistant with previous studies (Beketov et al., 2009; Bereswill et al., 2013; Hunt et al., 2017; Liess et al., 2008; Liess and von der Ohe, 2005; Münze et al., 2015; Orlinskiy et al., 2015). In the present study, neonicotinoid insecticides dominated the toxicity in agricultural streams. This is in accordance with several recent studies that reported effects of neonicotinoids on aquatic communities in agricultural streams (Becker and Liess, 2017; Münze et al., 2017; Shahid et al., 2018).

4.2. Adaptation to pesticides and consequences on the fitness

Our findings reveal that populations of *Gammarus pulex* in agricultural streams have aquired increased tolerance to the neonicotinoid insecticide clothianidin. Even low insecticide concentrations ($TU \leq -3$) were sufficient to produce more tolerant populations in the field. Similar results were reported by Shahid et al. (2018).

Populations from agricultural streams with increased insecticide tolerance showed decreased survival, per capita growth and mating. The observed effects were not only based on initial decrease in performance but they were persistent for at least four months (Table SI). However the observed effects became more pronounced and significant after three months of culture at pesticides free conditions. Persistent reduced performance observed in the present study may result in accumulative effects that get stronger until most individuals have died or stopped to reproduce. Persistent effects are an indicator for fitness costs from long-term adaptation rather than for delayed pesticide effects. Further, the persistent decrease in performance is of more concern than transient effects because it hinders population recovery and explains changes in the community composition over longer time scales at low concentrations. Our results indicate that in the field, even very low pesticide concentrations can considerably impair the performance of *G. pulex*, both directly through chronic effects and indirectly through fitness costs of genetic or physiological adaptation.

There are several investigations that report fitness costs of adaptation/increased tolerance to certain pesticides and metals in different organisms (Bach and Dahllof, 2012; Ffrench-Constant and Bass, 2017; Heim et al., 2018; Kliot and Ghanim, 2012; Xie and Klerks, 2004). It is suggested that molecular and physiological processes involved in tolerance development, and therefore also the associated fitness costs, depend on the intensity and duration of exposure (Amiard-Triquet et al., 2011). However, fitness costs of moderately increased tolerance caused by very low pesticide concentrations commonly observed in agricultural streams have never been investigated. Interference with energy allocation in an organism may impair life history traits such as survival, growth and reproduction (Novais et al., 2013). Therefore, the long-term negative effects investigated in *Gammarus pulex* might be due to their adaptation to pesticides and could potentially impact the performance of *Gammarus pulex* in natural ecosystems.

Additionally, delayed (chronic) effects from field exposure may have contributed to the decreased performance of populations from agricultural streams. Several studies have investigated long term effects after pulsed exposure to pesticides on macroinvertebrates under laboratory conditions (Abel and Garner, 1986; Barros et al., 2017; Rasmussen et al., 2017). For example, Cold and Forbes (2004) observed effects on survival, mating behavior, and reproductive output in *Gammarus pulex* exposed to 0.1‑0.6 µg/L of esfenvalerate for 1 h. These effects were even observed even after 2 weeks of pulse exposure, but much higher toxicity. Similarly, Liess and Schulz (1996) reported reduced survival and delays in emergence in the caddisfly, *Limnephilus lunatus*, during several weeks following 1h pulse exposure to fenvalerate. In another study, increase in the number of aborted broods and a decrease in viable offspring size in *Gammarus pulex* females were observed in response to zinc exposure (Maltby and Naylor, 1990).

However, all these effects were observed after pulse exposure to high pesticide concentrations that are known to cause acute effects after constant exposure. The present study suggests that long-term effects can be observed also after pulse exposure to much lower concentrations during run-off events. These effects might be attributed to resource allocation for different metabolic pr-ocesses in response to pesticide exposure (Baas et al., 2010; Jager et al., 2006). Resources used in detoxification processes significantly reduce the energy budget required for growth, fecundity, survival and reproduction.

5. Conclusions

Gammarus pulex shows moderate adaptation to pesticide pollution but also reduced long-term viability even at concentrations generally considered safe according to the European pesticide legislation. Long-term effects such as reduced survival, per capita growth, and reproduction may potentially impact the performance and ecosystem function of *Gammarus pulex*, such as leaf litter degradation, in the field. Therefore, these long-term impairments need to be considered in deriving save concentrations.

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Conflict of interest statement

The authors that they have no conflict of interest.

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