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Pesticide impacts on predator–prey interactions across two levels of organisation

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ABSTRACT

In this study, we aimed to evaluate the effects of a short pulse exposure of the pyrethroid lambda-cyhalothrin (LC) on the predator and anti-predator behaviour of the same species; *Gammarus pulex*. Predator behaviour, at the level of the individual, was studied in indoor microcosms using video tracking equipment during simultaneous exposure of the predator (*G. pulex*) and its prey (*Leuctra nigra*) during 90 min exposure of 1, 6.6 or 62.1 ng L^{-1} LC. During an initial 30 min of exposure, the predator and prey organisms were maintained physically separated, and the actual interaction was studied through the subsequent 60 min of exposure. The anti-predator behaviour of *G. pulex* (drift suppression in response to the presence of brown trout) was studied in outdoor stream channels during a 90 min pulse exposure to LC (7.4 or 79.5 ng L^{-1}) with, or without, brown trout. Based on survival curves for *L. nigra* we found that the mortality rate for *L. nigra* significantly decreased during exposure to 6.6 and 62.1 ng L^{-1} LC ($P < 0.05$ and $P < 0.001$, respectively). We found no significant effects suggesting that *G. pulex* was repelled by contaminated prey items ($P > 0.05$). We found that the exposure of *G. pulex* to 7.4 and 79.5 ng L^{-1} LC significantly increased drift (from ~0% to ~100% in both treatments; $P < 0.001$) independent of the presence of brown trout ($P < 0.05$). In other words, the natural anti-predator behaviour of *G. pulex* was overruled by the stress response to LC exposure increasing *G. pulex* predation risk from drift feeding brown trouts. Our results show that the anti-predator and predator behaviour of *G. pulex* were significantly changed during exposure to very low and environmentally realistic LC concentrations and exposure duration. The potential implications for the field scenario are discussed.

1. Introduction

The increasing contamination of freshwater ecosystems with numerous diffuse source synthetic pesticides is recognised as one of the most important stressors to freshwater ecosystems (Schwarzenbach et al., 2006). Particularly, the use of synthetic pyrethroid insecticides has raised much concern due to their high toxicity to non-target freshwater fauna (Schulz, 2004; Kuivila et al., 2012).

Traditionally, the ecotoxicity of specific chemical compounds is assessed by conducting standardised tests using selected model organisms (daphnia, algae and fish) and toxicity endpoints (i.e. mortality; Rand, 1995). However, effect thresholds for pesticides have been shown to be lower, in terms of changes in the macroinvertebrate community structure, in complex systems like mesocosms (Liess and Beketov, 2011) and natural streams (Schäfer et al., 2012) compared to what can be predicted from single species standard toxicity tests alone (despite the normally applied 100× safety factor). This may partly be explained by the influence of pesticide exposure on complex mechanisms and species interactions – effects that are not encompassed in single species toxicity tests. Thus, in order to understand pesticide effects at the level of ecosystems, detailed information is required on pesticide induced changes of species interactions incorporating different trophic levels. A specific interaction that can be affected by pesticide exposure is changed interactions between species and their natural predators.

Whereas several studies provide information on pesticide induced changes of predator–prey interactions, most of these studies were conducted using long exposure periods (>12 h; e.g. Ballesteros et al., 2009; Englert et al., 2012; Janssens and Stoks, 2012). Since highly hydrophobic compounds like synthetic pyrethroids ($\log K_{OW} > 5$) mainly occur in the aqueous form in short time intervals in the field, aquatic biota is exposed to them through the water phase for short periods only (Schulz, 2004). Subsequently, pyrethroids may sorb to particles, e.g. in the sediments providing

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a more chronic exposure route to benthic and epibenthic fauna (e.g. Kuivila et al., 2012). Nevertheless, the use of long term exposure with pyrethroids in the water phase is not realistic and may overestimate the effects of the water phase exposure in the field. Importantly, however, short-term exposure of freshwater macroinvertebrates to synthetic pyrethroids has been shown to induce long-term effects (see e.g. Liess and Schulz, 1996; Rasmussen et al., 2008).

The anti-predator behaviour of macroinvertebrate prey species has been shown to be affected during and after pulse exposure to pyrethroids (e.g. Schulz and Dabrowski, 2001; Reynaldi et al., 2011). The summed effect of pyrethroid exposure on prey populations may increase when the predator is less sensitive to the pesticide as indicated by Bundschuh et al. (2012). Nevertheless, effects of pesticides on populations can increase or decrease depending on the species involved, even when prey species are more tolerant.

The principal aim of this study was to assess the effects of a short-term exposure to the synthetic pyrethroid lambda-cyhalothrin (LC) at field-relevant concentrations on the freshwater amphipod *Gammarus pulex* (L.) in the role as predator and prey. The selection of *G. pulex* as model organism in this study is based on its wide distribution and often dominance in northern European lowland streams and its central role in stream food webs as both predator and prey (MacNeil et al., 1997). The brown trout (*Salmo trutta* L.) often co-exists with *G. pulex* and frequently preys upon drifting *G. pulex* (MacNeil et al., 1997). In consequence, especially large *G. pulex* are known to suppress drift activity in the presence of trout in order to avoid predation (Friberg et al., 1994). Conversely, *G. pulex* is very sensitive to pyrethroid exposure with active escape responses (catastrophic drift) at low concentrations, down to 1 ng L^{-1}, of LC (Nørum et al., 2010). The normal suppression of drift activity by *G. pulex* in the presence of trout may be overruled by pyrethroid exposure, hereby increasing the potential predation success of trout on drifting gammarids. *G. pulex* is characterised by high feeding plasticity (Kelly et al., 2002) mainly acting as a shredder, but it is also frequently found to prey upon other macroinvertebrate species (MacNeil et al., 1997). However, the stress imposed on gammarids by pyrethroid exposure significantly changes the behaviour of *G. pulex* (hyperactivity followed by decreased mobility or immobilisation; Nørum et al., 2010), which may affect the interaction between *G. pulex* and its prey. In addition, pyrethroid exposure may affect prey behaviour prompting additional changes in the predator–prey interactions. We used the stonefly *Leuctra nigra*, naturally belonging to the prey repertoire of *G. pulex* (MacNeil et al., 1997), as prey organism. We hypothesised that LC exposure would (1) initiate drift of *G. pulex* irrespective of the presence of trout increasing their proneness to predation by brown trout and (2) negatively influence predation rates of *G. pulex* on the stonefly *L. nigra*.

2. Materials and methods

2.1. Predator–prey interactions in the laboratory

G. pulex and *L. nigra* were collected in small Danish streams uncontaminated by pesticides (Lindved River, Funen for *G. pulex* and an unnamed stream in Velling Forest, Jutland for *L. nigra*). A few individuals of *G. pulex* were infected by an acanthocephalan parasite, e.g. *Pomphorhynchus laevis*, as evident by a bright orange line along the dorsal carapace, and since this parasite may alter the behaviour of *G. pulex* (Lagrue et al., 2007) these individuals were discarded.

The animals were temperature acclimated for 1 day in aerated stream water. *G. pulex* was acclimated at 15 ± 1 °C, while *L. nigra* was acclimated at 6 ± 1 °C in order to minimise mortality. Subsequently, the animals were transferred to 10-L polyethylene aquaria and acclimated for one week in aerated artificial freshwater (AFW) at constant temperature under a 12 h light:12 h dark regime. During the acclimation period the animals were fed ad libitum with leaf litter from the site of collection in order to avoid starvation and lost fitness as confounding factors in the experiment. AFW was used instead of stream water in order to minimise sorption of lambda-cyhalothrin to suspended nanoparticles. The composition of the AFW equalled the ISO 6431 test water of the OECD test guidelines (OECD, 2004). The species specific weight distributions were estimated from size measurements using previously published relations between the length of a morphological unit (length of 1st thoracic segment and width of head capsule for *G. pulex* and *L. nigra*, respectively) and the dry weight of the animals (Iversen and Jessen, 1977; Friberg et al., 2002). The estimated dry weight of *G. pulex* and *L. nigra* were 4.29 ± 0.10 mg and 0.29 ± 0.01 mg, respectively.

On the day prior to the exposure, individual *G. pulex* were transferred to glass Petri dishes (9 cm in diameter) and acclimated overnight at 15 ± 1 °C. On the day of exposure LC, dissolved in 10 μL of ethanol in 20 mL of AFW, was added to the Petri dishes (controls were exposed to 10 μL of ethanol and in 20 mL of AFW). The volume of 20 mL was chosen to ensure rapid mixing, and the liquid was added at a position as far away as possible from the animal. The final concentration of ethanol was 100 μL L^{-1}, which is in accordance with OECD test guidelines (OECD, 2000). Immediately after the addition of liquid, a polyethylene plastic tube (2.7 cm in diameter and open at both ends) was placed in the centre of each Petri dish, and a single *L. nigra* was transferred to the tube. In this way, the predator and the prey were physically separated during the initial 30 min of LC exposure. Subsequently, the plastic tubes were removed and the predator–prey interaction during a 60 min observation period of continued exposure was recorded using EthoVision Pro® (Noldus Information Technology, Holland) as described by Nørum et al. (2011). At the end of the experiment water was sampled for determination of LC concentration.

A total of 92 predator–prey pairs were divided into 5 treatment groups: a control group ($n = 24$), three groups exposed to 1 ng L^{-1} ($n = 25$), 10 ng L^{-1} ($n = 18$), and 100 ng L^{-1} ($n = 16$), and a group where only the *L. nigra* were preexposed to 100 ng L^{-1} for 30 min before being transferred to plastic tubes in Petri dishes containing uncontaminated water and unexposed *G. pulex* ($n = 9$) (all nominal concentrations). This final group was included to test if pre-exposure of the prey would have a repelling effect on *G. pulex*. In each round of video tracking, 16 predator–prey pairs were observed and the experiment was completed in 4 days.

2.2. Drift behaviour in stream channels

The study of drift behaviour was conducted in an outdoor stream channel facility in Lemming, Denmark (9° 40′, 56° 15′) consisting of 12 replicate channels being constantly supplied with uncontaminated groundwater (Supplementary material, Table B1). The individual stream channels were 4 m long, 10 cm wide and had a slope of 1%. Each channel was supplied with gravel (1–3 cm in diameter) along the first 3 metres (upstream). Moreover, larger stones (6–8 cm in diameter) were positioned every 30 cm, and two alder leaves (*Alnus glutinosa*) were mounted to each stone with cotton threads. The substrate was conditioned in the channels for 7 days prior to the experiment in order to establish microbial communities on substrates and leaves. A drift net was mounted at the downstream end of the channels for collection of drifting animals.

The experimental animals (*G. pulex*, $n = 30$) were collected in Lindved River (same locality as *G. pulex* for video tracking experiments) 24 h prior to the experiment and were released into the stream channels immediately after collection. Animals drifting out

of the stream channels within 1 h after release were reintroduced to the channels immediately. Furthermore, 1 h before exposure, the animals caught in the drift nets during the night were replaced by fresh individuals (50–60% of the animals).

We conducted two sets of experiments with or without 2 brown trouts caged in the water supply box (Supplementary material, Fig. B1). In each set of experiments, we used two LC treatments (10 ng L^{-1} and 100 ng L^{-1}; nominal concentrations) and controls ($n = 4$). The exposure period was 90 min and the LC solutions were released directly into each of the stream channels using peristaltic pumps. Water samples from each channel were collected just before terminating exposure (90 min) for determination of LC concentrations. The channels and substrates were changed between the two sets of experiments (with and without trout) to avoid cross contamination. All experiments were conducted in April 2005. The LC exposures were conducted at 10 AM where background drift of *G. pulex* is supposed to be minimal (Friberg et al., 1994). However, we did conduct an additional experiment at 10 PM, where drift is supposed to be maximal (Friberg et al., 1994). This was done in order to validate the effect of the applied level of trout odour on drift activity during the period of highest natural drift activity for *G. pulex* (Supplementary material, Fig. B2). During LC exposure, drift nets were emptied every 30 min. All drifted animals were preserved in 96% ethanol and size distributions were measured.

2.3. Quantification of LC exposure

The synthetic pyrethroid lambda-cyhalothrin was applied as analytical standards PESTANAL® (99.8% purity) and was purchased from Sigma–Aldrich (Selze, Germany). Dilutions of the pesticide were produced just before exposure, and the dilution series were based on 96% ethanol.

Immediately after collection of each water sample, we added a known concentration of internal standard (IS). The pyrethroid esfenvalerate was applied as internal standard since it has physicochemical properties similar to those of LC. The extraction of LC was conducted on a C18-column (Sep Pak Vac, 6cc, 1 g, C18 cartridges). The column was conditioned with 5 mL methanol and washed with 5 mL Milli-Q water. The sample was passed through the column at 20 kPa vacuum and subsequently washed with 5 mL Milli-Q water and dried for 2 min at 30–40 kPa vacuum. The LC samples were eluded with 4 mL methanol and re-concentrated by evaporation and re-dissolved in 0.3 mL 75% methanol.

We used a HPLC-MSD system consisting of a HP Series 1100 HPLC and a G1946A MSD quadropole mass spectrometer equipped with electrospray ionisation. A HPLC-column (C18, 150 mm × 2.1 mm, Phenomenex) with a guard column consisting of the same material was used for LC quantification. Using injected sample volumes of 50 μL and column temperature of 25 °C, the flow rate was 0.4 mL min^{-1}. We used: 10 mM ammoniumacetate:methanol, 990:10 (v:v) and 10 mM ammoniumacetate:methanol, 10:90 (v:v) as eluates A and B, respectively, and the elution gradient was: (time, % eluent B: (0 min, 75%); (3 min, 100%); (14 min, 100%); postrun time: 6 min, 25% eluent B). Mass spectrometer settings were: mode: ESI positive (SIM: m/z 467 for LC), (IS SIM: m/z 437). Drying gas temperature was 350 °C and flow 10 L min^{-1}. Nebulizer pressure was 30 psig and capillary voltage was 3500 V (Fragmentor: 50 V). The standard curve was based on the standards: 0.7, 3.5, 35.0, 70.0 and 350 ng L^{-1} injected IS. The limit of quantification of LC was 1 ng L^{-1} in the water samples. Actual exposure concentrations for the video tracking studies were 62.1 ng L^{-1} and 6.6 ng L^{-1} for the 100 ng L^{-1} and the 10 ng L^{-1} treatments, respectively, whereas the LC content in the 1 ng L^{-1} treatments was below the limit for quantification. For the stream channel studies on *G. pulex* drift activity, the quantified LC concentrations were 79.5 ng L^{-1} and 7.4 ng L^{-1} for the 100 ng L^{-1} and the 10 ng L^{-1} treatments, respectively. For clarity, we use measured concentrations in the remaining parts of the article.

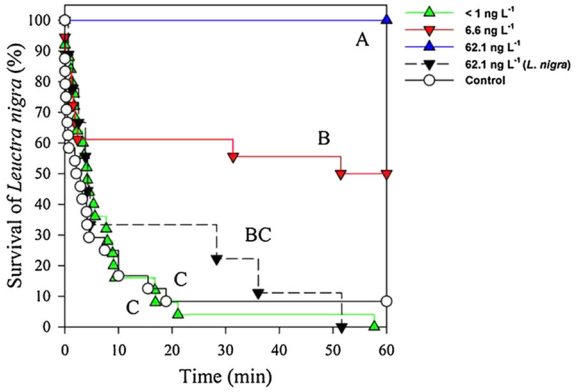

Fig. 1. Survival curves for *L. nigra* under predation by *G. pulex* during 60 min exposure to lambda-cyhalothrin (LC). Initially, the predator and the prey were physically separated through the first 30 min of exposure. Subsequently, the animals were allowed to interact and the predator–prey interaction was recorded through a 60 min observation period of continued exposure. In one treatment group, however, only *L. nigra* was exposed to LC (indicated in the legend). Capital letters indicate significantly different treatment groups.

2.4. Data analysis

For the predator–prey interactions between *G. pulex* and *L. nigra*, the survival curves of the prey in the 5 treatment groups were evaluated using a Kaplan–Meier log-rank survival analysis, followed by a Holm–Sidak adjusted multiple pairwise comparisons test. The statistical analysis was performed using SigmaPlot® 11.0 for Windows. A significance level of $\alpha = 0.05$ was used.

The drift activity of *G. pulex* in the presence or absence of trout odour during and after two different LC treatments and controls was analysed using a two-way Analysis of Variance ($n = 4$) with LC treatments and trout odour as effect parameters and summed drift activity as dependent variable. *A priori* we chose to only analyse summed drift activity after 90 min. Pairwise comparisons were performed using the Bonferroni adjusted Fisher's LSD in the cases of significant ANOVAs. Moreover, we a priori chose to compare the summed drift activity between the control treatments with and without trout odour with the Bonferroni adjusted Fisher's LSD. When necessary, data was transformed to obtain normality and homogeneity of variance. All statistical tests were conducted in SAS Enterprise Guide 4.3 using a significance level of $\alpha = 0.05$.

3. Results

We found a significant effect of LC treatments on the survival curves for *L. nigra* in the presence of *G. pulex* (Fig. 1; $P < 0.001$). The survival curves for the control group and the <1 ng L^{-1} LC treatment were significantly different from the 6.6 ng L^{-1} ($P = 0.02$ and $P = 0.007$, respectively) and the 62.1 ng L^{-1} LC treatments ($P < 0.0001$ for both), although no difference was observed between the control group and the <1 ng L^{-1} treatment ($P > 0.50$). Moreover, the survival curves were significantly different between the 6.6 ng L^{-1} and the 62.1 ng L^{-1} LC treatments ($P = 0.007$). Pre-exposure of *L. nigra* alone to 62.1 ng L^{-1} LC for 30 min prior to the 60 min predator–prey interaction period resulted in the survival curve being different from the 62.1 ng L^{-1} treatment ($P < 0.0001$), but not from the control group ($P > 0.50$), the <1 ng L^{-1} ($P > 0.50$), nor the 6.6 ng L^{-1} ($P > 0.10$) treatments.

Representative examples of interactions between *L. nigra* and *G. pulex* are given in Supplementary material A. In the control group and the <1 ng L^{-1} treatment the vast majority of prey were

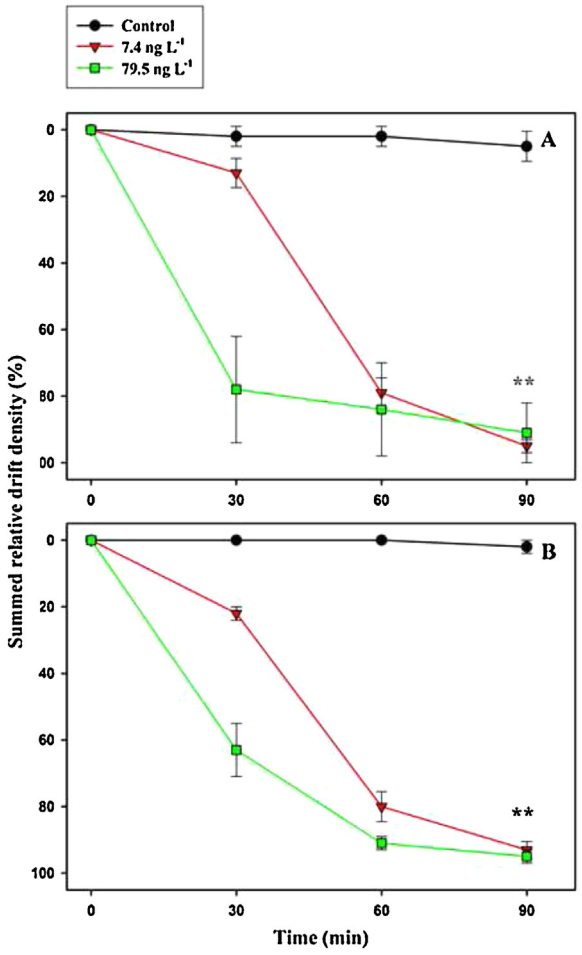

Fig. 2. Percentage drifted *G. pulex* as a function of time from initiated lambda-cyhalothrin (LC) exposure ($n = 4$) in the absence (A) or presence (B) of trout odour. The LC exposure was terminated after 90 min. Error bars represent standard error of the mean. Asterisks indicate significant differences compared to controls after 90 (**$P < 0.001$).

caught within a few minutes, mostly at the first encounter with the predator. *G. pulex* then typically spent 35–40 min on full prey consumption. When feeding was completed, *G. pulex* resumed locomotory behaviour. At 6.6 ng L^{-1} the variability in predatory behaviour increased. All *G. pulex* in this treatment displayed increased locomotory behaviour and although some *L. nigra* were caught and efficiently consumed, 50% (9 of 18) of the *L. nigra* were left untouched at the end of the 60 min interaction despite several encounters. Moreover, the time spent feeding varied highly and was often disrupted more quickly than in controls. At 62.1 ng L^{-1} all *G. pulex* were markedly hyperactive and the prey, *L. nigra*, was ignored completely despite repeated encounters.

We found a significant effect of LC treatment on the summed drift activity of *G. pulex* immediately after the 90 min LC exposure (Fig. 2; $P < 0.001$). There was no significant effect of trout odour, or interaction between trout odour and LC, after 90 min LC exposure (two-way ANOVA, $P > 0.05$). Moreover, the drift activity of *G. pulex* in the control treatments with and without trout odour was not significant ($P > 0.05$). However, the summed drift activity after the 90 min LC exposure was significantly increased in the 7.4 ng L^{-1} treatments ($P < 0.001$) and the 79.5 ng L^{-1} treatments ($P < 0.001$). Additional drift experiments conducted during night with and without trout odour showed that the drift activity of *G. pulex* was significantly reduced in the presence of trout odour during this normal active drift period (Supplementary material, Fig. B2; $P < 0.001$).

4. Discussion

During exposure to sublethal concentrations of LC the predator–prey interactions between *G. pulex* and *L. nigra* were significantly altered. The relative frequency of successful predation by *G. pulex* on *L. nigra* decreased from nearly 100% in the control and the <1 ng L^{-1} treatments to approximately 50% in the 6.6 ng L^{-1} treatment, and no predation was observed in the 62.1 ng L^{-1} treatment during the 60 min observation period. These findings probably reflect an increased stress response of *G. pulex* to increasing concentrations of LC prompting behavioural hyperactivity that overrules the natural instinct of catching the prey. Nørum et al. (2010) showed that the time for offset of hyperactivity for *G. pulex* in the video tracking arenas correspond to the offset of drift in outdoor experimental stream channels. In fact, Nørum et al. (2010) showed that a pulse exposure of 10 ng L^{-1} LC prompted active drift of all *G. pulex* in stream channels within 90 min. This additionally suggests that the 50% predation success observed in our study in the 6.6 ng L^{-1} treatment probably reflects the artificially promoted encounters of predator and prey organisms in the video tracking arena. We suggest that the 6.6 ng L^{-1} treatment was not fully sufficient for overruling the natural instinct of *G. pulex* to catch its prey when encountered. Supported by Nørum et al. (2010), we also observed hyperactivity in the <1 ng L^{-1} LC treatment, but the increased stress response in <1 ng L^{-1} treatments was not sufficiently strong to significantly suppress natural foraging behaviour of *G. pulex*.

The pre-exposure of *L. nigra* did not significantly change the predatory activity by unexposed *G. pulex* suggesting that there was no indirect repelling effect through the contamination of prey items which supports the findings of Reynaldi et al. (2011) and Janssens and Stoks (2012). However, through visual inspection it was apparent that a few of the pre-exposed *L. nigra* remained undetected comparatively longer than in the control group or the <1 ng L^{-1} treatment. These *L. nigra* were immobilised by the LC pre-exposure suggesting that pressure waves in the water caused by movement of the *L. nigra* to some extent may be used by *G. pulex* to support the detection of prey items. Interestingly, Lauridsen et al. (2006) and Rasmussen et al. (2012) reported that gammarids foraging on leaf material pre-exposed to pyrethroid insecticides showed reduced shredding activity through 7–26 days post-exposure due to probably a repelling effect of contaminated leaves. Since gammarids prefer food items with high nutritional value (such as other macroinvertebrates; Bundschuh et al., 2011), our findings could imply that the contamination of high quality food items with pyrethroid insecticides may not prompt a reduction in *G. pulex* ingestion rates similar to what has been observed for lower quality food items such as leaves. More research is needed to explore the mechanisms behind these observations.

The applied test organisms in our study have comparable sensitivities to pyrethroids (Nørum et al., 2010). However, considering that the physiological sensitivity of macroinvertebrate species to pyrethroids range several orders of magnitudes (Wogram and Liess, 2001), the responses in predator–prey interactions of other species strongly depend on the sensitivities of the predator and the prey organisms.

Due to high log K_{OW} of pyrethroids, the active ingredients are expected to be rapidly taken up by freshwater macroinvertebrates (Tang and Siegfried, 1995), and several authors have showed long term effects of short pulse exposures to pyrethroids using functional (Rasmussen et al., 2008) and life-cycle endpoints (Liess and Schulz, 1996; Schulz and Liess, 2000,2001). Moreover, Reynaldi

et al. (2011) found no effects of a 1 h pulse of fenvalerate on the predator–prey interactions of *Notonecta glauca* and *Culex pipiens* without applying a postexposure observation period. Since applying postexposure observation periods may increase the response sensitivity of predator–prey interactions to pyrethroid exposure we suggest that the true effect thresholds in our study may in fact be lower than our results suggest.

We found that the exposure with sublethal concentrations of LC significantly increased the drift activity of *G. pulex* during a 90 min exposure period, indicating that LC exposure prompted immediate active or passive drift initiation in accordance with previous findings (Liess, 1994; Heckmann and Friberg, 2005). More importantly, we found that the increased drift rates as response to LC exposure was maintained irrespective of the presence of trout. Moreover, from night drift experiments we found that the applied level of trout odour in the experimental streams were sufficient to significantly suppress the natural drift activity of *G. pulex* (Supplementary material, Fig. B2). Thus, we conclude that the natural behavioural response of *G. pulex* to the presence of trout (decreasing their drift activity; Friberg et al., 1994) was overruled by the response to LC exposure. In fact, virtually all *G. pulex* drifted out of the stream mesocosms in both LC treatments suggesting that significant effect concentrations are below the lowest concentration (7.4 ng L^{-1}) applied in our study. Since the behavioural adaptation of *G. pulex* to the presence of trout is probably an active anti-predator response minimising the risk of predation, we suggest that the increase in drift activity prompted by LC exposure can increase the predation rate of brown trout on *G. pulex* in the field. Resident trouts in streams receiving pyrethroid insecticides are also exposed to the pesticides, which may influence their predatory behaviour. However, the 24 h LC50 for young rainbow trouts (*Oncorhychus mykiss*) exposed to LC is 41.7 μg L^{-1} (Kucukbay et al., 2009) and it is therefore highly unlikely that short pulses of LC, with concentrations ranging from 0.01 to 0.1 μg L^{-1}, induce significant behavioural changes to the taxonomically related brown trout. Moreover, Schulz and Dabrowski (2001) showed that the predation success of a drift-feeding fish (*Galaxias zebratus*) on mayfly nymphs (Genus: *Baetis*) increased significantly during a short pulse exposure to another synthetic pyrethroid; fenvalerate (0.2 μg L^{-1}), because the mayflies displayed decreased ability to maintain a fully coordinated swimming behaviour. A similar effect may occur in *G. pulex* due to its high sensitivity to pyrethroid insecticides which may further increase the success rate of trout predation on *G. pulex* during pyrethroid exposure.

Our results show that the natural predator and predator-avoidance behaviour of *G. pulex* is overruled by stress responses to short pulses of low and environmentally realistic concentrations of the synthetic pyrethroid lambda-cyhalothrin (e.g. Feo et al., 2010; Weston and Lydy, 2012). Considering the pivotal role of *G. pulex* in stream ecosystems, the observed changes of the predator and predator-avoidance behaviour during LC exposure could influence the population density of *G. pulex* and their role in the food web. Surely, a key question when using a mesocosm set-up with limited complexity is to what extent these artificial conditions mimic natural and more complex systems. However, our findings add some mechanistic understanding of how species interactions can be modified by pyrethroid exposure and, more importantly, how sensitive such endpoints can be. In addition, our study also provides some insight into the reasons why field-based macroinvertebrate community endpoints are more sensitive than what could be expected from single species standard ecotoxicity tests alone (even though a 100 fold safety margin is maintained; Schäfer et al., 2012).

Overall, effects of environmentally realistic pesticide exposure on predator–prey interactions have received limited attention in freshwater ecotoxicology, but our, and recent studies of other authors (e.g. Bundschuh et al., 2012; Englert et al., 2012; Pestana et al., 2009; Relyea and Hoverman, 2006), document the urgent need for more research with long-term endpoints and more complex set-ups in order to comprehend the potential effects of pesticides across different levels of organisation with the ecosystems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquatox.2013.06.019.

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