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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