Impact of lambda-cyhalothrin on a macroinvertebrate assemblage in outdoor experimental channels: Implications for ecosystem functioning

Jes Jessen Rasmussen^{a,c}, Nikolai Friberg^{b,*}, Søren E. Larsen^c

^a University of Aarhus, Department of Biological Sciences, Ole Worms Allé, Building 1135, 8000 Aarhus C, Denmark

^b Macaulay Land Use Research Institute, Catchment Management Group, Craigiebuckler, Aberdeen AB15 8QH, UK

^c University of Aarhus, National Environmental Research Institute, Department of Freshwater Ecology, Vejløvej 25, DK-8600 Silkeborg, Denmark

ARTICLE INFO

Article history:

Received 24 July 2008

Received in revised form 29 August 2008

Accepted 4 September 2008

Keywords:

Stream macroinvertebrates

Pyrethroids

Drift

Ecosystem function

Functional feeding groups

ABSTRACT

In this study, the impact of a single pulse of the pyrethroid lambda-cyhalothrin was tested on a macroinvertebrate assemblage consisting of *Gammarus pulex*, *Leuctra nigra*, *Heptagenia sulphurea* and *Ancylus fluviatilis* in outdoor experimental stream channels. Channels (4 m long, 0.1 m wide) were groundwater fed and had natural substratum. Macroinvertebrates were exposed to 10.65 or 106.5 ng L⁻¹ lambda cyhalothrin for 90 min in the laboratory and after 24 h introduced to the experimental stream channels with four replicates of each treatment and controls. Drift samples were taken with 24-h interval for 10 days and behaviour of drifted macroinvertebrates was assessed. Microalgae biomass was measured on days 1, 5, 8 and 10 along with leaf litter decomposition using leaf packs of beech (*Fagus sylvatica*). Numbers of drifting *G. pulex* and *L. nigra* with reduced mobility increased significantly with concentration of lambda-cyhalothrin. Increase of algal biomass was significantly greater in stream channels with macroinvertebrates exposed to 106.5 ng L⁻¹ compared to controls and 10.65 ng L⁻¹ treatments. Accrual of microalgal biomass was significantly higher in the high concentration treatment and decomposition of leaf litter was significantly greater in control channels compared to channels with exposed macroinvertebrates. This study may apply valuable knowledge to the understanding and assessment of how pyrethroids impact ecosystem functioning in streams.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Pesticides have a wide range of impacts on the aquatic biota (Larson et al., 1997) and in streams direct impacts have been found at all trophic levels ranging from algae to fish (Davies and Cook, 1993; Kreutzweiser et al., 1995; Guasch et al., 1997). Pesticides are widely used in crop protection and are transported to streams by a range of different routes including wind drift, run-off or point sources (Hill et al., 1994). The major contributor to pesticide contamination is reported by several studies to be drainage water/sub-surface run-off, which strongly coincides with high levels of precipitation (Mathiessen et al., 1995; Liess and Schulz, 1999; Schulz and Liess, 1999a). Both dissolved and particle bound pesticides are transported from the soil surface or subsurface to stream recipients (Schulz et al., 1998) with pesticides having low water solubility (e.g. pyrethroids) generally dissipating very rapidly from the dissolved phase (Schroer et al., 2003). Depending on different physical and chemical characteristics of the receiving stream as well as

physical and chemical properties of pesticides, both particle-bound and dissolved pesticides give rise to short (1–2 h) pulses of acute contamination followed by series of smaller pulses during the subsequent 24 h (Liess et al., 1999; Kronvang et al., 2004). In contrast, pesticides bound to the sediment can be retained in the stream ecosystem for longer periods (weeks to months) after the actual exposure incident has occurred (Kronvang et al., 2003; Friberg et al., 2003).

In Denmark 62% of the total area (43,000 km²) is used as agricultural land. Total length of natural and artificial streams is 60,000 km, the majority of which are small in size (<2 m wide). In Denmark approximately 80% of the total agricultural land is sprayed with pyrethroids (Danish Environmental Protection Agency, 2003). The combination of extensive use of pyrethroids and the close connectivity between land and stream, enhanced artificially by drainage and narrow buffer strips, makes the potential risk of pyrethroid exposure extremely high (Friberg et al., 2003). Until now, only few records of detection of pyrethroids in the stream water phase exist, which is likely explained by the episodic nature of pesticide transport to streams, the rapid dissipation from the water phase and infrequent sampling (Lauridsen et al., 2006). Stream water concentrations of pyrethroids more than 0.1 µg L⁻¹ were detected in

* Corresponding author. Tel.: +44 1224 498200.

E-mail address: n.friberg@macaulay.ac.uk (N. Friberg).

20% of the water samples collected in relation to high precipitation incidents in three Danish streams draining agricultural areas with up to $66 \mu\text{g L}^{-1}$ found on one occasion (Wiggers, 1999). Likewise, sediment samples in 30 Danish streams have revealed that the pyrethroid lambda-cyhalothrin was the most frequently found insecticide (7%) and in concentrations up to 20 ng g^{-1} sediment (Kronvang et al., 2003; Friberg et al., 2003).

Pyrethroids primarily have an effect on the nervous system by decreasing the decay of the action potential (e.g. Clark and Matsumura, 1982; Narahashi et al., 1995). The toxicological impact of pyrethroids has been intensively tested in the laboratory and in field/mesocosm setups on various non-target species with stream macroinvertebrates attracting considerable attention as they respond strongly to pyrethroid exposure (e.g. Anderson, 1989; Schulz and Liess, 1999b). Many previous studies of the toxicological impact of pyrethroids on stream macroinvertebrates have used mortality, immobilization and offset of catastrophic drift as end-points (e.g. Farmer et al., 1995; Schulz and Liess, 1999b; Heckmann and Friberg, 2004). Despite the brief episodic occurrence and low actual concentrations of pyrethroids in natural streams, the highly toxic nature of pyrethroids may produce long term effects not readily observable. Pyrethroid contamination has the potential to produce fundamental impacts on ecosystem structure, function and biotic interactions due to a combination of direct and indirect effects (Schulz and Liess, 1999b). Leaf litter breakdown rates are frequently used proxies for ecosystem function (Gessner and Chauvet, 2002) and have been shown to decrease with elevated concentrations of pesticides in the field (Schäfer et al., 2007). A laboratory study has, furthermore, shown a reduction in the leaf shredding activity of the trichopteran *Sericostoma personatum* and the amphipod *Gammarus pulex* when exposed to lambda-cyhalothrin in environmentally realistic concentrations (Lauridsen et al., 2006). These findings indicate that pyrethroids may cause indirect effects in more complex experimental systems or natural streams, and could potentially impair ecosystem functioning.

The aim of the present study was to investigate in outdoor experimental channels: (1) direct effects of lambda-cyhalothrin on mortality, drift and behaviour on a macroinvertebrates assemblage, representing different life histories and feeding functionalities, and relate these effects to (2) proxies of ecosystem function represented by accrual of microalgal biomass (grazing pressure) and decomposition rates of leaf litter (shredder activity).

2. Materials and methods

2.1. Channel setup

The experiment was conducted in an outdoor stream channel facility in Lemming, Denmark ($9^\circ 40'$, $56^\circ 15'$) and carried out in April 2005. The setup consisted of 12 replicated stream channels, which were constantly supplied with groundwater. Using groundwater instead of surface water has the advantage of reducing risks of contamination and a constant chemical composition over time. Chemical properties of the water used were typical for Denmark with fairly high levels of nitrogen but low concentrations of phosphorous (Table 1). The water was oxidized before being led through a distribution box which supplied 12 experiment channels with equal amounts of water (Table 1). The stream channels were 4 m long and 0.1 m wide with a slope of 1%, which is comparable to a riffle section in a small Danish stream. Prior to the experiment they were stocked with coarse gravel/small pebbles (1–3 cm in diameter) that had been conditioned in nutrient enriched water to allow establishment of a dense biofilm. Seven cobbles (10–15 cm) were equally spaced in each channel to increase retention and create suitable habitats. Mean water residence time was 52 s and mean

Table 1

Chemical and physical parameters for stream channel water

Chemical parameters	Value/concentration
NH_4 (mg L^{-1})	0.009
$\text{NO}_2 + \text{NO}_3$ (mg L^{-1})	2.720
Total N (mg L^{-1})	2.830
PO_4 (mg L^{-1})	0.003
Total P (mg L^{-1})	0.003
Total Fe (mg L^{-1})	0.005
pH	7.0
Physical parameters	
Discharge per stream channel (L min^{-1})	3.5
Mean water resident time per stream channel (s)	51.6 ± 2.7
Mean water velocity (m s^{-1})	0.0679 ± 0.003
Water temperature (range) ($^\circ\text{C}$)	6.48–7.96

water velocity 0.07 m s^{-1} which would be typical for low flow conditions in small streams (Table 1). A Stowaway Tidbit temperature logger was positioned in the downstream end of the substratum sections, and temperature fluctuation was measured for 24 h (Table 1).

2.2. Macroinvertebrate assemblage

The macroinvertebrate assemblage used was composed of four species: *Gammarus pulex* (amphipod), *Leuctra nigra* (stonefly), *Heptagenia sulphurea* (mayfly) and *Ancylus fluviatilis* (gastropod). Selection of these macroinvertebrates was based on a series of criteria. They represent some of the most dominant taxonomic groups of macroinvertebrates in Denmark (Dall and Lindegaard, 1995). They differ in life history and feeding functionality encompassing scrapers of algae and biofilm (*Heptagenia*, *Ancylus*), shredders of leaf materials (*Gammarus*, *Leuctra*) and predators of other macroinvertebrates (*Gammarus*) (Møhlenberg et al., 2004). Individuals used for experimentation were collected at local stream sites immediately (days) before being exposed.

2.3. Pesticide and exposure

Applied concentrations of lambda-cyhalothrin were selected based on peak concentrations of pyrethroids found in water samples from Danish agricultural streams during high flow events (Wiggers, 1999 in Introduction). A dilution series was produced in ethanol (96%) to achieve intended concentrations of lambda-cyhalothrin. Exposure was conducted in plastic containers with 10 L groundwater. Lambda-cyhalothrin was applied in 5 ml ethanol in all exposure containers. 5 mL ethanol was applied in control containers as well. Macroinvertebrates were introduced to exposure and control containers (lightly aerated at 4°C) immediately after application of ethanol (controls) or the lambda-cyhalothrin solution (exposure). Intended concentrations of lambda-cyhalothrin were 10.65 ng L^{-1} and 106.5 ng L^{-1} ($n=4$) and the exposure period was 90 min. Water from exposure containers was collected immediately after the exposure period and analyzed for actual concentrations of lambda-cyhalothrin. The basic principle of the applied method used to quantify lambda-cyhalothrin concentrations is based on solid phase extraction followed by solution concentration in the process of evaporation and quantification using reverse phase HPLC-MS (Nørum et al., 2006). Actual exposure concentrations were 92 ng L^{-1} for the 106.5 ng L^{-1} treatment while concentrations were below detection limit (10 ng L^{-1}) in the 10.65 ng L^{-1} treatment. After exposure, macroinvertebrates were transferred to aerated plastic containers with uncontaminated groundwater (4°C) for 24 h with access to food (leaf litter and pebbles with biofilm). After this period macroinvertebrates were transported to

the outdoor research facility and distributed to channels. Initial species numbers in each channel for *G. pulex*, *L. nigra*, *H. sulphurea*, *A. fluviatilis* were 65, 45, 16 and 25, corresponding to the densities of 433, 300, 107 and 167 individuals m^{-2} , respectively. Each treatment group (control, 10.65 and 106.5 ng L^{-1}) was replicated in four stream channels.

2.4. Macroinvertebrates in drift

Macroinvertebrates were monitored in the stream channels for 10 consecutive days. At the end of each channel a net (mesh size 0.5 mm) was mounted to collect drift samples and nets were emptied every day at 10 a.m. in the experimental period. All macroinvertebrates caught were counted and their behaviour was assessed using three categories: normal activity (preserved escape response by physical touch), reduced activity (restricted escape response by physical touch) and dead. All macroinvertebrates alive were reintroduced into the respective stream channels. At the end of the experiment, all remaining substratum was preserved in 96% ethanol and later sorted for macroinvertebrates.

2.5. Microalgae accrual and leaf litter decomposition

Increase of algal biomass reflects grazing activity of macroinvertebrates in this simplified community. In this study we used chlorophyll a density as a measure for algal coverage of the substratum. The gravel/pebble substrate was collected from each channel on experimental days 1 (prior to the introduction of macroinvertebrates) 5, 8 and 10 for chlorophyll a analysis. Chlorophyll was extracted in ethanol and the absorbance at 665 nm was measured in a PharmaSpec UV-1700 spectrophotometer. Total chlorophyll a was calculated from the projected area of each collected gravel/pebble.

Leaf pack weight loss reflects shredding activity and as such is a measure for decomposition of coarse particulate organic material (CPOM). Five leaf packs were placed evenly spaced upstream of cobbles to secure that they would keep position. Each leaf pack was constituted of 10 conditioned beech leaves (*Fagus sylvatica*) collected from a small groundwater fed first order stream in Velling Forest, Denmark. The catchment for this stream is entirely old beech forest, thus the collected leaves have not been exposed to environmental toxins prior to the experiment. Initial dry weight (DW) was established by drying 15 randomly selected beech leaves to a constant weight at 60 °C and then weighing on a Mettler Toledo XP-204 balance with three decimals accuracy. After the last emptying of driftnets on day 10, all leaf packs were retrieved and macroinvertebrates were removed and preserved in 96% ethanol. Final DW of each leaf pack was established using the procedure described above.

2.6. Macroinvertebrate size estimations

The size of different morphological parts was measured on all macroinvertebrates and DW was calculated using established relationships between the size of a morphological measure (e.g. the width of head capsules) and their DW (Zelinka and Marvan, 1976; Iversen and Jessen, 1977; Friberg et al., 2002). For *A. fluviatilis* it was necessary to establish a new length/DW relationship by collecting additional individuals in the field and measuring length of shells followed by individual DW estimation. For all species DW was estimated using simple linear regression and was 4.45 ± 0.10 mg for *G. pulex*, 0.29 ± 0.007 mg for *L. nigra*, 1.64 ± 0.07 mg for *H. sulphurea* and 4.35 ± 0.25 mg for *A. fluviatilis*.

2.7. Statistics

The primary analytical approach of drift, macroinvertebrate activity, microalgae accrual and leaf litter decomposition data were ANOVA and repeated measures ANOVA since response variables were observed over time (Littell et al., 1996). Specifically, drift data were analysed by a repeated mixed effect model (Littell et al., 1996) with pesticide treatment as fixed effect, channels as a random effect and day as repeated measure. The variance structure was modelled by a compound symmetry variance matrix. Pairwise tests were done by least square means and *t*-tests (SAS Institute Inc., 1989). The analysis of algae was done using exactly the same methods as for the drift data, but the variance structure was modelled by an unstructured variance matrix. The leaf pack data was analyzed by a mixed effect model with pesticide treatment as fixed effect and channels as random effect. Pairwise tests were done by least square means and *t*-tests.

3. Results

3.1. Channel macroinvertebrate numbers

The number of macroinvertebrates did not decline significantly ($P > 0.05$) during the experimental period in any treatment group or channel as mortality was low and all individuals alive caught in drift were reintroduced. Average total number of *G. pulex* ranged from 61 individuals in 106.5 ng L^{-1} treatment to 63 individuals in controls, from 42 (106.5 ng L^{-1} treatment) to 44 (controls) for *L. nigra*, from 14 (106.5 ng L^{-1} treatment) to 15 (controls) for *H. sulphurea* and with *A. fluviatilis* numbers being constant at 25 individuals across treatments and controls.

3.2. Macroinvertebrate drift

The proportion of *G. pulex* in drift increased for all groups (control, 10.65 and 106.5 ng L^{-1}) after 4 days reaching a plateau for the remaining part of the experimental period (Fig. 1a). Drift in control channels was significantly higher than both treatment groups ($t = 9.54$, $P < 0.0001$ and $t = 16.70$, $P < 0.0001$, respectively). Between treatment groups drift was significantly higher in channels with macroinvertebrates exposed to 10.65 ng L^{-1} compared with 106.5 ng L^{-1} ($t = 7.16$, $P < 0.0001$).

As for *G. pulex* drift of *L. nigra* increased after 4 days but no consistent trend was evident in drift pattern among the three groups (Fig. 1b). Only few *H. sulphurea* drifted and there were no significant differences in drift pattern between treatment groups (data not shown). No *A. fluviatilis* drifted in the experimental period.

3.3. Activity of macroinvertebrates

The proportion of *G. pulex* that maintained a normal activity level decreased with the concentration of lambda-cyhalothrin (Fig. 2a) which in 106.5 ng L^{-1} treatment corresponded to 3% of the total number of individuals. The number of *G. pulex* individuals showing reduced mobility increased slightly with concentration of lambda-cyhalothrin. Compared to controls, the proportion of *G. pulex* with normal activity level was significantly lower in both treatments ($t = 8.46$, $P < 0.0001$ and $t = 15.68$, $P < 0.0001$, respectively). Between treatments the proportion of individuals with normal activity level was significantly lower in the 106.5 ng L^{-1} treatment compared to 10.65 ng L^{-1} ($t = 7.22$, $P < 0.0001$). Activity level of *G. pulex* was significantly reduced in treatment channels ($F_{9,81} = 20.21$, $P < 0.0001$) and with significant interaction between time and concentration ($F_{18,81} = 7.11$, $P < 0.0001$) (data not shown).

Fig. 1. The average proportion of *G. pulex* (a) and *L. nigrata* (b) in drift during the experimental period (10 days) $\pm$ S.E. in stream channels with individuals treated with 0 (control), 10.65 and 106.5 ng L⁻¹ lambda-cyhalothrin. Each treatment and control with four replicates.

with the proportion of *G. pulex* with reduced mobility significantly increasing through the experimental period ($F_{9,81} = 3.26$, $P = 0.0020$).

The proportion of *L. nigrata* in drift with normal activity declined with treatments while the proportion of individuals showing reduced mobility increased (Fig. 2b). The proportion of individuals with reduced activity level was significantly lower in the 106.5 ng L⁻¹ treatment compared to controls ($t = -4.14$, $P = 0.0025$) and the 10.65 ng L⁻¹ treatment ($t = -2.54$, $P = 0.0317$). There was a significant interaction between time and concentration for activity level ($F_{18,81} = 1.99$, $P = 0.019$) (data not shown).

Despite that only few *H. sulphurea* were observed in drift, activity level in the 106.5 ng L⁻¹ treatments was reduced compared to the 10.65 ng L⁻¹ treatments and controls (Fig. 2c). The proportion of individuals with normal activity was significantly different between groups of treatment. ($F_{2,9} = 5.73$, $P = 0.024$), and there was a significant effect of the time of sampling ($F_{9,81} = 2.22$, $P = 0.029$). No *A. fluviatilis* was found in drift during the experiment (data not shown).

3.4. Microalgal biomass accrual

Algal biomass increased during the experimental period in all groups. There was a significant effect of treatment and time of sampling on algal biomass ($F_{2,9} = 5.09$, $P = 0.033$ and $F_{2,9} = 8.46$, $P = 0.0086$, respectively). Treatment with 106.5 ng L⁻¹ lambda-cyhalothrin had a strong effect on the grazers' ability to reduce algal biomass. Average initial chlorophyll a density was 3.57 mg m⁻². In

channels with macroinvertebrates exposed to 106.5 ng L⁻¹ lambda-cyhalothrin chlorophyll a density increased to 16.15 mg m⁻², while the increment during the 10-day experimental period chlorophyll a merely increased to 6.25 and 4.30 mg m⁻² in 10.65 ng L⁻¹ treatment and controls, respectively (Fig. 3). Algal biomass was significantly higher in channels with macroinvertebrates exposed to 106.5 ng L⁻¹ compared to the 10.65 ng L⁻¹ treatment and controls ($t = -2.49$, $P = 0.035$ and $t = -2.98$, $P = 0.016$, respectively). The increase of algal biomass in the 10.65 ng L⁻¹ treatments was not significantly greater than in control channels.

3.5. Leaf litter decomposition

Total decomposition of leaf packs was significantly higher in control channels compared to channels with treated macroinvertebrates ($t = -2.98$, $P = 0.016$ and $t = -2.49$, $P = 0.035$). Leaf pack weight loss was 0.17 g DW in control channels, three times greater than 0.05 and 0.06 g DW in 10.65 and 106.5 ng L⁻¹ treatments, respectively (Fig. 4). The difference in leaf pack weight loss between the two treated groups was not significant ($P > 0.05$).

4. Discussion

Effects of pesticide contamination on community and ecosystem level in streams have previously been observed at environmentally realistic concentration levels (e.g. Wallace et al., 1989; Sibley et al., 1991; Schulz and Liess, 1999b). Other more recent studies have found various indirect effects of pesticide exposure in addition to the more frequently used end points such as mortality or exclusion of species (Lauridsen et al., 2006; Nørum et al., 2006; Schäfer et al., 2007). In this study several species were allowed to interact in a seminatural system and the results support previous findings of direct and indirect effects of pyrethroids on macroinvertebrates in running waters. Density of species in the mesocosm remained approximately constant during the experiment because of the reintroduction of all living individuals caught in drift. Thus differences in leaf decomposition rate and algal grazing activity cannot be attributed to decreasing species densities according to loss of macroinvertebrates from the stream channels.

During the 10-day experimental period, only *G. pulex* significantly changed drift pattern compared to the control group. Drift significantly decreased with lambda-cyhalothrin concentration, while there were no significant changes in drift activity between treatment groups for *L. nigrata*, *H. sulphurea* and *A. fluviatilis*. An increase in drift rate for *G. pulex* in controls and 10.65 ng L⁻¹ treatments and for *L. nigrata* in all treatments was observed on day 5. No significant external disturbance could explain this change but it might reflect a change in weather to more overcast conditions promoting drift activity in general (see e.g. Allan, 1995). The initially lower drift activity for both *G. pulex* and *L. nigrata* and the subsequent higher but stable activity level for *G. pulex* might indicate an acclimation period after their introduction into the channels. Previous studies have shown that the primary response of *G. pulex* and other macroinvertebrates is to enter catastrophic drift when exposed to pyrethroids (e.g. Muirhead-Thomsen, 1978; Davies and Cook, 1993; Heckmann and Friberg, 2004; Nørum et al., 2006). The observed decrease in post exposure drift rate in *G. pulex* with pesticide treatment might be ascribed to a reduction in activity level that make them less prone to enter drift. These findings are in accordance with previous video tracking recovery studies (Nørum et al., 2006), where *G. pulex* was exposed to 100 ng L⁻¹ lambda-cyhalothrin for 4.5 h. *G. pulex* here showed reduced mobility in a subsequent period of 1–3 days, and mortality increased in the entire recovery period of 21 days.

Fig. 2. The condition of (a) *G. pulex*, (b) *L. nigra* and (c) *H. sulphurea* in drift during the experimental period (10 days) ± S.E. average and S.E. are based on 10 observations per channel with four replicates for each treatment group 0 (control), 10.65 and 106.5 ng L⁻¹ lambda-cyhalothrin. All individuals were visually classified being with normal activity (preserved escape response by physical touch), reduced activity (restricted escape response by physical touch) and dead.

According to functional grouping of the four macroinvertebrate species in the mesocosm community, *A. fluviatilis* is the most significant and efficient grazer followed by *H. sulphurea* (Elliott et al., 1988; Dall and Lindegaard, 1995). However, both *G. pulex* and *L. nigra* can feed on benthic algae if the resource is readily accessible (Marchant, 1981; Henderson et al., 1990).

The significant increase in total algal biomass in stream channels with macroinvertebrates treated with 106.5 ng L⁻¹ compared with control and the 10.65 ng L⁻¹ treatment might indicate that the ability of the macroinvertebrate assemblage to regulate total algal biomass was altered. In accordance to this Kjeldsen (1996) found that algal biomass on rocks frequently rinsed with the pyrethroid

permethrin was higher than that of rocks rinsed with water, caused by a release of macroinvertebrate grazing pressure. Our setup did not directly allow us to assess how feeding activity of each individual species was affected by the exposure. However, considering that neither drift activity nor behaviour of *A. fluviatilis* appeared to be altered in any way and the reported low sensitivity of molluscs to pyrethroids (Schroer et al., 2003), it is unlikely that this species was responsible for the change in grazing pressure. With regard to *H. sulphurea* too few individuals were caught in drift and assessed to establish if this species was affected by the treatment to a degree that would impact feeding efficiency. In contrast, both *G. pulex* and *L. nigra* were clearly affected and it seems likely that these two

Fig. 3. Changes of algal biomass during the experimental period (10 days). Gravel used for chlorophyll a analyses was gathered on day 5, 8 and 10 and for reference on day 1. Each mark represents average ± S.E. for each treatment group (0 (control), 10.65 and 106.5 ng L⁻¹ lambda-cyhalothrin).

Fig. 4. Weight loss (g DW) in leaf packs during the experimental period (10 days) ± S.E. average and S.E. are based on 5 leaf packs per streamchannel with four replicates for each treatment group (0, 10.65 and 106.5 ng L⁻¹ lambda-cyhalothrin).

species were primarily responsible for changes in grazing pressure, indicating that they might be more efficient grazers of microalgae than normally believed.

In this study treatment with lambda-cyhalothrin had a pronounced effect on macroinvertebrate shredding activity. Decomposition of leaf packs was significantly lower in channels with treated macroinvertebrates compared to controls but there were no clear dose-response effect. *G. pulex* and *L. nigra* dominated the community in number and density. Both species act primarily as shredders and feed upon CPOM in natural ecosystems (Iversen, 1988). The activity of individuals of both species in drift was reduced in both 10.65 and 106.5 ng L⁻¹ treatments though the assessed condition of *L. nigra* was based on relatively few numbers. These results are supported by other studies using video tracking which have revealed long term reduction of mobility in *G. pulex* after exposure to 10 ng L⁻¹ lambda-cyhalothrin, cypermethrin or esfenvalerate in the laboratory (Nørum and Bjerregaard, 2003; Nørum et al., 2006).

Our findings suggest that *G. pulex* and *L. nigra* are highly sensitive to this pyrethroid and that effects can be seen not only in form of readily induced short-term behavioural changes such as hypermobility, but also as longer term effects with subsequent reduced mobility and possibly feeding activity. This contention is supported by Møhlenberg et al. (2004) who found reductions in shredding activity for *G. pulex* exposed to 0.02 µg L⁻¹ esfenvalerate for 30 min. Furthermore *L. nigra* and *G. pulex* are the two most sensitive species towards pyrethroids in this simplified macroinvertebrate community (e.g. Anderson and Shubat, 1984; Giddings et al., 2001; Schroer et al., 2003). The lack of a clear dose-response correlation regarding shredding activity between the 10.65 and 106.5 ng L⁻¹ treatments could be explained by the relatively short experimental period. Nørum et al. (2006) studied predator-prey interactions between *G. pulex* and *L. nigra* impacted by lambda-cyhalothrin and found the strongest dose-response correlation between 1 and 10 ng L⁻¹. Thus the observed effect of lambda-cyhalothrin concentration on shredding activity, and maybe grazing pressure as well, would possibly have been stronger with a longer experimental period.

The exposure scenario we used was mimicking a run-off event where pesticides recently applied, either dissolved or bound to particles, enters the stream ecosystem in periods with high precipitation. Several Danish studies have shown this scenario provides the highest peak concentrations with regard to pyrethroids and occurs more frequently under natural conditions than other routes of exposure such as spray drift (e.g. Wiggers, 1999; Kronvang et al., 2004).

A key question when utilising a mesocosm approach is to what extent these artificial environments mimic natural conditions, i.e. their realism. Limited biological complexity or scale-dependent behaviour of component species could limit the relevance and applicability to real systems (Cooper and Barmuta, 1993). We used concentrations of lambda-cyhalothrin in a range that have been observed in Danish streams (Wiggers, 1999) and exposed macroinvertebrates to a short 90 min pulse mimicking realistic exposure conditions under high run-off events. As we exposed macroinvertebrates in the laboratory without the combined effect of increased water velocities that would occur under in-stream conditions, our experimental design might underestimate the negative impact of lambda-cyhalothrin on macroinvertebrates in real exposure scenarios. In contrast, we might have overestimated impacts on ecosystem function as there is likely to be a greater functional redundancy in natural systems compared with our simplified four-species assemblage. However, negative impacts of pyrethroids on both grazing and leaf litter processing has been reported from studies of natural streams (Kjeldsen, 1996; Schäfer et al., 2007)

and our experiment clearly supports the hypothesis that lambda-cyhalothrin potentially can change ecosystem function.

Acknowledgements

The authors gratefully acknowledge the support of the Danish Environmental Protection Agency, J. Kristensen and T.K. Jensen for field assistance and U. Nørum for pesticide analyses. Moreover, we would like to thank T. Riis and A. Vinten for valuable comments on an earlier draft of the manuscript. N. Friberg was partly sponsored by the Scottish Executive Environment and Rural Affairs Department (WP3.4).

References

- Allan, J.D., 1995. Stream Ecology. Chapman & Hall, London.
- Anderson, R.L., 1989. Toxicity of synthetic pyrethroids to freshwater invertebrates. Environ. Toxicol. Chem. 8, 403–410.
- Anderson, R.L., Shubat, P., 1984. Toxicity of flucythrinate to *Gammarus lacustris* (Amphipoda), *Pteronarcys dorsata* (Plecoptera) and *Brachycentrus americanus* (Trichoptera)—importance of exposure duration. Environ. Pollut. A: Ecol. Biol. 35, 353–365.
- Clark, J.M., Matsumura, F., 1982. Two different types of inhibitory effect of pyrethroids on nerve Ca- and Mg ATPase in the squid, *Loligo paeli*. Pestic. Biochem. Physiol. 4, 232–238.
- Cooper, S.D., Barmuta, L.A., 1993. Field experiments in biomonitoring. In: Rosenberg, D.M., Resh, V.H. (Eds.), Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman & Hall, London, pp. 341–399.
- Dall, P., Lindegaard, C., 1995. A Review of Danish Freshwater Invertebrates for Assessment of Pollution in Lakes and Streams. Ferskvandsbiologisk Laboratorium, Københavns Universitet (in Danish).
- Danish Environmental Protection Agency, 2003. Pesticide Statistics 2002. Danish Environmental Protection Agency, Orientering fra Miljøstyrelsen 5. Copenhagen, Denmark (in Danish).
- Davies, P.E., Cook, L.S.J., 1993. Catastrophic macroinvertebrate drift and sublethal effects on brown trout, *Salmo trutta*, caused by cypermethrin spraying on a Tasmanian stream. Aquat. Toxicol. 27, 201–224.
- Elliott, J.M., Humpesch, U.H., Macan, T.T., 1988. Larvae of the British Ephemeroptera: A Key with Ecological Notes. FBA Scientific Publication no. 49, Ambleside, Cumbria, UK.
- Farmer, D., Hill, I.R., Maund, S.J., 1995. A comparison of the fate and effects of two pyrethroid insecticides (lambda-cyhalothrin and cypermethrin) in pond mesocosms. Ecotoxicology 4, 219–244.
- Friberg, N., Larsen, A.D., Rodkjær, A., Thomsen, A.G., 2002. Shredder guilds in three Danish forest streams contrasting in forest type. Arch. Hydrobiol. 153, 197–215.
- Friberg, N., Lindstrøm, M., Kronvang, B., Larsen, S.E., 2003. Macroinvertebrate/sediment relationships along a pesticide gradient in Danish streams. Hydrobiologia 494, 103–110.
- Gessner, M.O., Chauvet, E., 2002. A case for using litter breakdown to assess functional stream integrity. Ecol. Appl. 12, 498–510.
- Giddings, J.M., Solomon, K.R., Maund, S.J., 2001. Probabilistic risk assessment of cotton pyrethroids: II. Aquatic mesocosm and field studies. Environ. Toxicol. Chem. 20, 660–668.
- Guasch, H., Munoz, N., Roses, N., Sabater, S., 1997. Changes in atrazine toxicity throughout successions of stream periphyton communities. J. Appl. Phycol. 9, 137–146.
- Heckmann, L.H., Friberg, N., 2004. Macroinvertebrate community response to pulse exposure with the insecticide lambda-cyhalothrin using in-stream mesocosms. Environ. Toxicol. Chem. 24, 582–590.
- Henderson, J., Hildrew, A.G., Townsend, C.R., 1990. Detritivorous stoneflies in an iron-rich stream: food and feeding. I: I.C. Campbell (red.) Mayflies and stoneflies. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Hill, I.R., Travis, K.Z., Ekoniak, P., 1994. Spray drift and runoff simulations of foliar-applied pyrethroids to aquatic mesocosms: rates, frequencies and methods. In: Grane, R.L., Kennedy, J.H., Rodgers, J.H. (Eds.), Aquatic Mesocosm Studies in Ecological Risk Assessment. Lewis, Boca Raton, FL, USA, pp. 201–239.
- Iversen, T.M., 1988. Secondary production and trophic relationships in a spring invertebrate community. Limnol. Oceanogr. 33, 582–592.
- Iversen, T.M., Jessen, J., 1977. Life-cycle, drift and production of *Gammarus pulex* (L.) (Amphipoda) in a Danish spring. Freshwater Biol. 7, 287–296.
- Kjeldsen, K., 1996. Regulation of algal biomass in a small lowland stream: field experiments on the role of invertebrate grazing, phosphorous and irradiance. Freshwater Biol. 36, 535–546.
- Kreutzweiser, D.P., Capell, S.S., Sousa, B.C., 1995. Hexazone effects on stream periphyton and invertebrate communities. Environ. Toxicol. Chem. 14, 1521–1527.
- Kronvang, B., Laubel, A., Larsen, S.E., Friberg, N., 2003. Pesticides and heavy metals in Danish streambed sediment. Hydrobiologia 494, 93–101.
- Kronvang, B., Strøm, H.L., Hoffmann, C.C., Laubel, A., Friberg, N., 2004. Subsurface tile drainage loss of modern pesticides: field experiment results. Water Sci. Technol. 49, 139–148.

- Larson, D.J., Capel, P.D., Majewski, M.S., 1997. Pesticides in Surface Water: Distribution, Trends and Governing Factors. Pesticides in Hydrological Systems, vol. 3. Ann Arbor Press, Michigan.
- Lauridsen, R.B., Kronvang, B., Friberg, N., 2006. Occurrence and impact of sediment-bound pyrethroids in Danish streams and their impact on ecosystem function. *Water Air Soil Pollut.* 6, 423–432.
- Liess, M., Schulz, R., 1999. Linking insecticide contamination and population response in an agricultural stream. *Environ. Toxicol. Chem.* 18, 1948–1955.
- Liess, M., Schulz, R., Liess, M.H.D., Rother, B., Kreuzig, R., 1999. Determination of insecticide contamination in agricultural headwater streams. *Water Res.* 33, 239–247.
- Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., 1996. SAS[®] System for Mixed Models. SAS Institute Inc., Cary, NC, 633 p.
- Marchant, R., 1981. The ecology of Gammarus in running water. In: Lock, M.A., Williams, D.D. (Eds.), *Perspectives in Running Water Ecology*. Plenum Press, New York, USA, pp. 225–250.
- Mathiessen, P., Sheahan, D., Harrison, R., Kirby, M., Rycroft, R., Turnbull, A., Volkner, V., Williams, R., 1995. Use of a *Gammarus pulex* bioassay to measure the effects of transient carbofuran runoff from farmland. *Ecotoxicol. Environ. Saf.* 30, 111–119.
- Møhlenberg, F., Kaas, H., Schlüter, L., Gustavson, K., Andersen, T.T., Forbes, V., Cold, A., Friberg, N., Larsen, S.E., Lauridsen, R.B., 2004. Effects of biocides and pesticides on flora and fauna in streams. *Bekæmpelsesmiddel-forskning fra Miljøstyrelsen* no. 82 (in Danish).
- Muirhead-Thomsen, R.C., 1978. Lethal and behavioral impact of permethrin (NRDC 143) on selected stream macroinvertebrates. *Mosq. News* 38, 185–190.
- Narahashi, T., Carter, D.B., Frey, J., Ginsburg, K., Hamilton, B.J., Nagata, K., Roy, M.L., Song, J., Tatebayashi, H., 1995. Sodium channels and GABA_A receptor-channel complex as targets of environmental toxicants. *Toxicol. Lett.* 82/83, 239–245.
- Nørum, U., Bjerregaard, P., 2003. Mobility and behaviour of freshwater invertebrates as bio-marker for pesticide exposure and -effect. *Bekæmpelsesmiddelforskning fra Miljøstyrelsen* no. 75 (in Danish).
- Nørum, U., Bjerregaard, P., Larsen, S.E., Friberg, N., 2006. Effects of pulse exposure of pyrethroids on stream invertebrates. *Bekæmpelsesmiddelforskning fra Miljøstyrelsen* no. 102 (in Danish).
- SAS Institute Inc., 1989. SAS/STAT[®] Users Guide, Version 6, vol. 2, fourth ed. SAS Institute Inc., Cary, NC, 846 p.
- Schäfer, R.B., Caquet, T., Siimes, K., Mueller, R., Lagadic, L., Liess, M., 2007. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. *Sci. Total Environ.* 382, 272–285.
- Schroer, A.F.W., Belgers, J.D.M., Brock, T.C.M., Matser, A.M., Maund, S.J., Van den Brink, P.J., 2003. Comparison of laboratory single species and field population-level effects of the pyrethroid insecticide λ -cyhalothrin on freshwater invertebrates. *Arch. Environ. Contam. Toxicol.* 46, 324–335.
- Schulz, R., Liess, M., 1999a. Validity and ecological relevance of an active in situ bioassay using *Gammarus pulex* and *Limnephilus lunatus*. *Environ. Toxicol. Chem.* 18, 2243–2250.
- Schulz, R., Liess, M., 1999b. A field study of the effects of agricultural derived insecticide input on stream macroinvertebrate dynamics. *Aquat. Toxicol.* 46, 155–176.
- Schulz, R., Hauschild, M., Ebeling, M., Nanko-Drees, J., Wogram, J., Liess, M., 1998. A qualitative field method for monitoring pesticides in the edge-of-field runoff. *Chemosphere* 36, 3071–3082.
- Sibley, P.K., Kaushik, N.K., Kreutzweiser, D.P., 1991. Impact of a pulse application of permethrin on the macroinvertebrate community of a headwater stream. *Environ. Pollut.* 70, 35–55.
- Wallace, J.B., Lugthart, G.J., Cuffney, T.F., Schurr, G.A., 1989. The impact of repeated insecticidal treatments on drift and benthos of a headwater stream. *Hydrobiologia* 179, 135–147.
- Wiggers, L., 1999. Pesticides in Streams, Springs and Lakes in the County of Aarhus. Aarhus Amt, Aarhus, Denmark (in Danish).
- Zelinka, M., Marvan, P., 1976. Notes to estimating production of zoobenthos. *Folia Fac. Sci. Nat. Univ. Purkynianae Brunensis* 17, Biology 58, 1–53.