# Effects of repeated insecticide pulses on macroinvertebrate drift in indoor stream mesocosms

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## ABSTRACT

Pesticide contaminations via run-off or spray drift have been reported to result in the mass drift of macroinvertebrates as well as causing structural and functional changes of the corresponding stream sections. However, pesticide pulses in the field are associated with sudden increases in flow velocity, water turbidity, and changes in water temperature, which can also induce drift. Only through replicated community testing under highly controlled conditions can these effects be disentangled. In a stream mesocosm study, 12-h pulses of 12 µg/L imidacloprid were set three times at weekly intervals and are considered a "pulse series". Two pulse series of this neonicotinoid insecticide were run in both spring and summer with 4 treatment and 4 control stream mesocosms used in each pulse series. Prior to the start of the mesocosm experiment, both pulse concentration and duration had been screened for drift responses in larval Baetidae, Chironomidae and adult *Gammarus roeseli* in laboratory experiments. In the subsequent mesocosm study, each pulse caused a pronounced increase in the drift of insect larvae and gammarids. The drift response was taxon-specific, which was related to preferred habitat and exposure to other stressors like current velocity, in addition to imidacloprid sensitivity. Activity measurements employing a Multispecies Freshwater Biomonitor® revealed that in *Baetis* sp. the diurnal activity pattern became more pronounced even 12 h after the pulse though with slightly decreased mean physical activity. Adult *G. roeseli* showed a drastic pulse by pulse decrease in physical activity which after the 3rd pulse lasted longer than 24 h. In conclusion, drift is a sensitive, ecologically relevant endpoint and should be regarded when a specific risk assessment for lotic surface waters is done, e.g. in the context of a spatially explicit risk assessment.

## 1. Introduction

Macroinvertebrate drift is a complex behaviour defined as passive and active downstream transport and has often been reported from field observations for a number of macroinvertebrate taxa (Elliott, 2002a,b,c; Svedsen et al., 2004). Drift allows macroinvertebrates to escape from unfavourable physical, chemical and biological conditions and to colonize new habitats. The entry into drift may be active e.g. as an escape from predators, or passive as dislodgement during phases of both high activity and increasing water flow (Elliott, 2002a). Drift can be induced by natural factors such as photoperiod, rapid changes in temperature, turbidity, predation pressure or competition (Allan, 1995), but also by anthropogenic factors such as pesticide contamination (Wallace et al., 1989). Both drift intensity (i.e. number of organisms drifting

through the cross-sectional stream area per time unit, Pegel, 1980) and the distance travelled may increase during and after exposure to these stressors.

In ecotoxicological studies, mass drift of macroinvertebrates in the course of pronounced pesticide contaminations has often been reported (e.g., Burdick et al., 1960; Wallace and Hynes, 1975; Dosdall and Lehmkuhl, 1989; Sebastien et al., 1989; Zwick, 1992) and there is evidence for the ecological importance of catastrophic drift events (Sibley et al., 1991; Breneman and Pontasch, 1994). The potential to initiate drift has been demonstrated for many chemicals and was detected at concentrations that are one order of magnitude or less below acute toxicity (see, e.g., Beketov and Liess, 2008a). Drift events may result in structural and functional changes of the corresponding stream sections. In univoltine species with long ontogenetic development of several years and in non-volant species such drift events may induce considerable long-term shifts in the aquatic community (Zwick, 1992). Among other things, mass development of filamentous green algae (Yasuno et al., 1982; Wallace et al., 1991a; Zwick, 1992) and drastic reductions in foliage

decomposition due to decreasing numbers of shedders (Wallace et al., 1991b) may occur. Macroinvertebrate reduction in the course of pesticide-induced drift events, which may even mean the temporary disappearance of certain species in the polluted stream stretches (Zwick, 1992; Nørum et al., 2010), may also affect species composition in the corresponding catchment area. Consequently, Hall et al. (1980) proposed using invertebrate drift as an index of human disturbance. Indeed, monitoring amphipod drift has been demonstrated to be a good indicator for assessing the impact of insecticides, especially pyrethroids (e.g., Kingsbury, 1976; Liess, 1994; Lauridsen and Friberg, 2005). In general, pesticide pulses entering streams via inflow from drainage, ground water, wastewater treatment plants, and run-off from rainfall (see review by Schulz, 2008) are associated with sudden increase in flow velocity, water turbidity, and changes in water temperature. These confounding factors have drift-inducing potential themselves and therefore have to be disentangled from the pesticide effect (Schulz and Liess, 1999). The complex interactions within pulse events may be the reason why macroinvertebrate downstream drift has not yet been regarded in the EU ecotoxicological environmental risk assessment. Replicated community testing under highly controlled conditions in stream mesocosms may be one solution to disentangle pesticide-induced effects from other factors.

The effects of repeated short term pulses of the neonicotinoid insecticide imidacloprid on the endpoints invertebrate drift and behaviour were studied in an indoor stream mesocosm experiment under highly controlled conditions. Two application series (spring and summer) of 3 successive 12 h pulses of 12 µg/L imidacloprid were run to simulate repeated imidacloprid applications (Mohr et al., 2012). Imidacloprid was chosen as test substance based on the findings of a pre-study, in which 11 selected pesticides had been screened for drift-initiating effects in *Gammarus pulex*, *Simulium latigonium*, and *Baetis rhodani* (Beketov and Liess, 2008a). It is a neonicotinoid insecticide, which is neurotoxic and used on fruit and vegetable crops against sucking insects and as biocide for lawn, in stables, and against pet lice. It is highly photodegradable and also highly soluble in water ($\log_{kow} = 0.57$ g/L) which was necessary to avoid memory effects in the mesocosms between pulses. The chosen pulse concentration of 12 µg/L is well within the range of environmental imidacloprid concentrations in surface waters ranging from 1 to 320 µg/L (Canadian Council of Ministers of the Environment, 2007; Jemec et al., 2007; Tennekes, 2010). It is also close to the globally predicted environmental concentrations (PEC) after regular treatment of apple trees with 200 g/L imidacloprid, which amounts to 8 µg active substance/L (FOCUS, 2001, scenario R3 stream, Bologna, Italy). Moreover, it was in the range of the drift initiating concentrations found by Beketov and Liess (2008a) and therefore promising for seeing a drift effect.

The study reported here was part of a project concerning the ecological effects of repeated short term insecticide pulses in flowing waters. The first part dealt with effects on community structure and insect emergence (Mohr et al., 2012). The paper at hand comprised investigations on drift behaviour and indoor stream mesocosm drift experiments. Besides investigating invertebrate drift, which was measured by means of driftnets, physical activity in larval *Baetis* sp. and adult *Gammarus roeseli* was recorded in the stream mesocosms during and after the pulses employing a Multispecies Freshwater Biomonitor® (MFB, LimCo International). Prior to the start of the mesocosm study, pulse concentration and pulse duration were screened for drift responses of selected taxa in a laboratory drift meter (Werth and Marten, 2007) in order to test whether the animals can still actively regain the substratum during pulses at the given concentrations of 12 µg/L and the pulse duration of 12 h (cp. Elliott, 2002b). This information was needed to make sure that the drift net catches in the mesocosms were representative and could be used for the calculation of drift
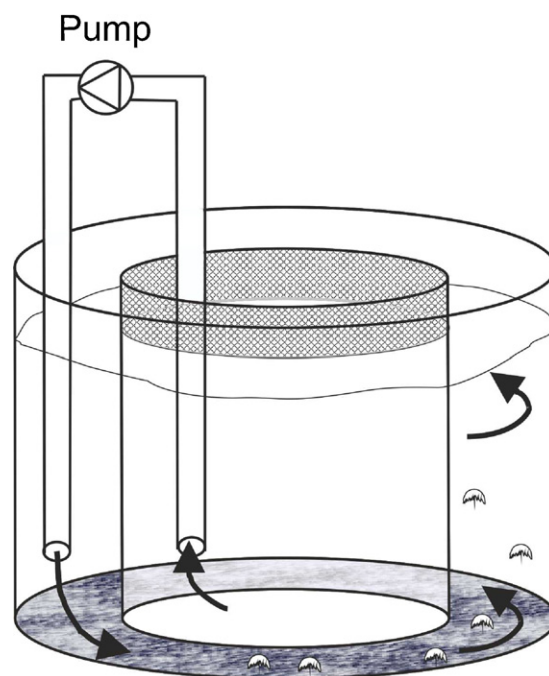


**Fig. 1.** Diagram of the carousel drift meter after Werth and Marten (2007).

intensity. For the subsequent mesocosm study, the questions to be answered were: Which taxa are the most sensitive to imidacloprid based on drift? What are the influences of repeated pulses of imidacloprid on the behaviour and drift of macroinvertebrates? Is drift a relevant endpoint for pesticide risk assessment? If so, is drift a suitable measured endpoint, or should it be considered in the risk assessment, i.e. by means of a safety factor?

## 2. Materials and methods

### 2.1. Preliminary drift meter investigations in the laboratory

Late instars of *Cloeon dipterum* (Ephemeroptera, Baetidae) were taken from outdoor artificial streams, late instars of *Chironomus riparius* (Diptera, Chironomidae) from a laboratory culture, *G. pulex* and both juvenile and adult *G. roeseli* (both Crustacea, Gammaridae) from a reference stream to test their behaviour in a drift meter prior to the start of the mesocosm study. The carrousel drift meter of Werth and Marten (2007) was slightly modified (Fig. 1): The corridor between the two cylindrical glass jars of 22.5 and 13.5 cm was filled with 2 cm of washed sand. The ripples that were formed by the water current on the sand corridor between the inner and the outer container allowed the animals to choose their favoured flow regime. After dosing 12 µg/L imidacloprid, the behaviour (duration of drift in the water column and on the sediment surface, positive rheotaxis, exposure to the water current, and active body movements) of 10 specimens per trial was observed directly every half hour not only for 12 but also for 26 h. Light was provided by a tubular fluorescent light (Osram LF72), which produced 10.2 µmol/m²/s PhAR (Photosynthetically Active Radiation) at the water surface. The light regime was 16 h light:8 h dark. Water temperature was 17.7 ± 1 °C. Maximum current velocity at the water surface was 20 cm/s. The current speed close to the sand bottom was ≪10 cm/s.

### 2.2. Mesocosm study

#### 2.2.1. Mesocosm features

The study was carried out in the indoor stream mesocosms (FSA) of the German Federal Environment Agency. Technical details on

the stream mesocosm system can be obtained from Mohr et al. (2005) and the homepage (last accessed 5 January 2012). The 8 circular indoor streams were each 75 m long and had 42 m$^2$ of sand bottom. The streams were operated at 0.2 m water depth and 0.1 cm/s flow velocity. Each stream contained 4 pools sections that were planted with *Sparganium emersum* to add both habitat and flow diversity. The light regime was adjusted to the season. At the latitude of mesocosms in Berlin (52.396702°N) the days around summer solstice allowed for only about 5 h of complete darkness disregarding the phase of the moon and light pollution (Perkin et al., 2011). Dawn and dusk and its changes in the course of the season were regarded by the incidence of natural light through the windows in the saw-tooth roof of the mesocosm hall, which are oriented towards the north. For more details concerning physical environment and the general operation conditions during the mesocosm study see Mohr et al. (2012).

### 2.2.2. Stocking

In the mesocosm study, a representative part of the community was transferred from a reference stream into the artificial indoor streams. The reference stream was the Barolder Fließ (Brandenburg, Germany), a sublacustrine creek, which is sandy, macrophyte rich, partly shaded, β-mesosaprobic and unpolluted (for details see Mohr et al., 2012). The water temperature is similar to that in the FSA and does not exceed 25 °C. 144 straw bags – 6 mm mesh opening – were used as attraction, transport and stocking devices. The bags were exposed in the reference stream one or two weeks. One week prior to the start of the pulse series, the bags were removed by means of a fine-meshed net, transported humid but not immersed to the FSA, immediately transferred into the artificial streams and opened so that the straw could aggregate in 8 cm deep × 1 m long wells, which had been created perpendicular to the flow direction. Thus the distribution of straw was similar in all streams, leaving 3 different strata to be sampled in each stream: the wall, sand, and straw compartments. Prior to each imidacloprid pulse, the bottom coverage with straw was mapped in each stream and the straw, sand and wall sections were sampled at 5 randomly selected points by means of tube corers and wall scrapers for determination of the actual population sizes (for details see Mohr et al., 2012). The samples of each stratum were pooled and fixed in 80% ethanol for identification and counting. After the first series of 3 pulses starting in April, the streams were re-stocked with another 144 straw bags from the reference stream one week prior to the start of the second series of 3 imidacloprid pulses in mid-June.

### 2.2.3. Pulse simulation

Each pulse series consisted of three successive 12 h pulses one week apart with the insecticide imidacloprid and was conducted in 4 stream mesocosms. Four further mesocosms served as controls. For all six 12 h pulses, the same concentration 12 μg/L was chosen. For pulse simulation, 1 treatment and 1 control stream were dosed simultaneously in the evening (8 p.m. CET) employing multi-channel tube pumps, which had been adjusted in flow rate for dosing to just one single circuit of stream water operated in circular mode. Application in the dark is common practice in agriculture when using pesticides like imidacloprid, which are highly prone to photolysis. In the case of imidacloprid farmers are also advised to apply late in the evening or early in the morning to reduce potential damage to honey bees (http://celdf.org/downloads/Chemical%20Trespass%20and%20HONEY_BEES.pdf, http://www.fs.fed.us/foresthealth/pesticide/pdfs/122805_Imidacloprid.pdf, last accessed in March 2012). Moreover, this dosing also kept the imidacloprid concentration constant during the pulses without any re-dosing since the artificial light in the experimental hall was free of UV-radiation and photolysis of imidacloprid was thereby negligible.

The imidacloprid stock solution was mixed by strong aeration 1 m downstream of the tube pump inlet. The common nontoxic tracer uranine was used to control for both complete mixing of imidacloprid in the stream water and complete removal at the end of the pulse. It was measured on-line by means of a SCUFA fluorometer (Turner Designs, Sunnyvale, USA). The controls were pulsed with tracer, only. After 12 h exposure time on the next morning both streams were flushed with 30 m$^3$ of water of similar quality and temperature as in the streams in order to completely remove the contaminated water body. A 0.5 mm net at the end of the outlet pipe prevented the fauna from passing through the effluent while a net with a mesh opening of 1.4 mm in the stream prevented the outlet pipe net from clogging. Sampling was conducted weekly and included macrozoobenthos, water for chemical analysis of both imidacloprid and nutrients, and emergence in 4 traps of 1 m$^2$ at each stream. The endpoints were species diversity, species abundance, emergence (see Mohr et al., 2012), drift, and behaviour (this paper). Species diversity and abundance were determined from the macrozoobenthic samples which were taken every week 6 to 4 h before the imidacloprid pulses (for details see Mohr et al., 2012).

### 2.2.4. Imidacloprid analysis

Achieving both the nominal imidacloprid concentration of 12 μg/L and the complete removal of imidacloprid at the end of the pulse after flushing was checked on the basis of single 1 L water samples. The samples were taken directly upstream from the on-line measuring devices in each stream and for each pulse 11.5 h after setting the pulses and 1 day after the contaminated water had been flushed out. After solid phase extraction (SPE) employing modified polystyrene-divinylbenzene resin (ENV + 200 mg/6 mL, IST Biotage) imidacloprid was derived for gas chromatographic–mass spectrometric (GC–MS) analysis. Following MacDonald and Meyer (1998), pentafluorobenzoyl chloride (CAS-No. 2251-50-5) was used instead of hepta-fluorobutyric anhydride and pyridine. The internal standard was imidacloprid-D4. Squalane (CAS-No. 111-01-3) and dibromo octafluoro biphenyl (DBOFB, CAS-No. 10386-84-2) served as volume control standards. Derived extracts were analysed in a GC–MS system HP 6890/5973 (Hewlett-Packard) with split–splitless injector (250 °C) in a 50 m capillary column coated with a 0.25 μm film of 95% polydimethyl siloxane and 5% of phenyl siloxane (CP Sil 8 CB, Varian). The transfer line was at 280 °C. Helium served as carrier gas and was supplied in constant flow mode. Monitoring of the mass chromatograms $m/z$ 405, 407, and 212 for imidacloprid, and those of 409, 411, and 216 for imidacloprid-D4 was done in selected ion mode (SIM).

### 2.2.5. Analysis of physico-chemical parameters

On-line measurements included dissolved oxygen, pH, water temperature, and water conductivity (see Mohr et al., 2005). Moreover, physico-chemical standard water parameters were monitored on a weekly basis employing the corresponding DIN EN ISO standard techniques. Alkalinity was measured using the titrimetric method according to DIN EN ISO 9963-1 (1996) and Gran plots according to Sigg and Stumm (1989). Phosphate (PO4-P), ammonia (NH4-N), nitrogen oxides (NOX) and silicate (SiO$_2$) were analysed employing colorimetric continuous flow analysis CFA (San++, SKALAR, NL; Bendschneider and Robinson, 1952; Mullin and Riley, 1955; Murphy and Riley, 1962; Chaney and Marbach, 1962; Wood et al., 1967; DIN 38405 Part 21, 1990; DIN EN ISO 13395, 1996; DIN EN ISO 15681, 2004; DIN EN ISO 11732, 2005).

### 2.2.6. Drift measurements

Drift before, during, and after the pulses was detected employing two drift nets with brown plastic openings (15 cm × 7.5 cm, mesh opening in the funnel 283 μm, mesh opening in the codend 150 μm, white mesh, 140 cm length) in each mesocosm, which

were placed in the middle of the stream bottom above the sediment surface in front of the 2nd and the 4th pool section (distance between nets = 20 m) with opening in flow direction. On 3 occasions, 2 additional drift nets per stream were used, which were placed behind the pool sections 1 and 3. Catches in the week prior to insecticide pulsing included the comparison of night and day catches in all mesocosms as a reference for unpolluted conditions. After imidacloprid dosing, each drift net was checked 3 times: at the end of each pulse (1st night), at the end of the following day (1st day), and on the second morning (2nd night). Specimens of *G. roeseli* ≤ 3.8 mm total length were counted separately and are referred to in the following as 'early juveniles'. For data analysis, the 3 large size classes were pooled and are referred to in the following as '*G. roeseli* > 3.8 mm'. Because the population sizes in the 8 mesocosms were very similar (Mohr et al., 2012), effects of imidacloprid on macroinvertebrate drift were calculated as quotient of all driftnet catches in the treatments and all driftnet catches in the corresponding control stream. Significant differences ($p < 0.05$) between treatment and control catches of driftnets, which were synchronously exposed in the same stream mesocosms, and between replicates were tested pulse by pulse with the Wilcoxon signed-rank test (Lozan and Kausch, 1998).

### 2.2.7. Activity measurements in the mesocosms

Physical activity of selected macroinvertebrate species during and after the pulses was monitored on-line employing an 8 channel Multispecies Freshwater Biomonitor® (MFB, LimCo International, Konstanz, Germany). This device allows for synchronous activity measurements of single specimens caged in chambers (inner diameter 1.8 cm, length 6 cm) which are equipped with quadropole sensors (Gerhardt et al., 1998) and were placed in the water column of the stream mesocosms. Test organisms were adults and juveniles of *G. roeseli* and stage 5 larvae of *Baetis fuscatus* from the reference stream. Test duration was 2 days at maximum. The animals were not fed during this period. For evaluation, the mean activity of the invertebrates in each of the 8 chambers was plotted against time for each hour and the trend line of the controls and treatments compared.

## 3. Results

### 3.1. Preliminary drift meter investigations in the laboratory

Even after 26 h of exposure, all *C. dipterum* in the carrousel drift meter were alive and had not passed over to completely passive drift but drifted only occasionally a few decimetres at all water levels and then returned to the ground. Transfer into uncontaminated water resulted in the test organisms regaining their mobility within a few minutes. The same drift behaviour and recovery was true for both juvenile and adult *G. roeseli*. In contrast, the chironomid *C. riparius*, which was still active after 12 h, was almost immobile after 26 h. It also took the chironomids a few hours to regain some mobility in uncontaminated water.

### 3.2. Mesocosm study in spring and summer

Mean water temperature during the first series in May was 16.3 °C (±3 °C) and 19.3 °C (±3 °C) during the second series in June (Fig. 2). Diurnal variations matched the ones found in the sublacustric reference stream (Hensel and Kiel, 2006). The diurnal pattern in oxygen saturation around 90% (75.6–100%) and pH around 8.0 (7.5–8.2) realistically reflected the autotrophic activity of the systems. Water turbidity was low with a range of 0.6–1.5 nephelometric turbidity units (NTU) at the start of pulse series 1 and 0.4–1.6 NTU at the end of pulse series 2 and always allowed for direct observations of animal behaviour at the stream bottom.
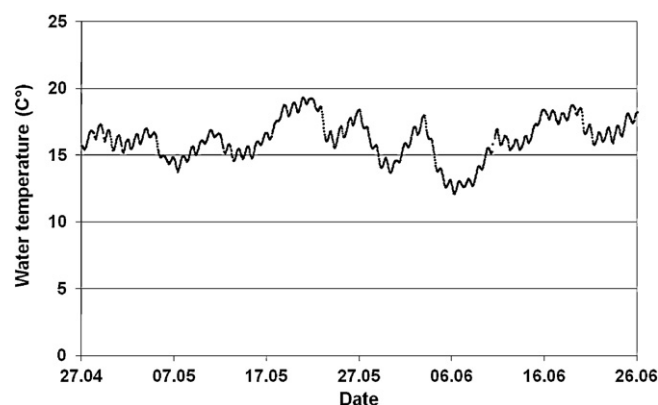


**Fig. 2.** Means of water temperature on the basis of hourly online measurements in the artificial streams.

Mean concentration of nitrate ($NO_3$-N) was 0.102 mg/L, of phosphate ($PO_4$-P) 0.007 mg/L, of silicate ($SiO_2$) 2.6 mg/L, and of total organic carbon (TOC) 2.3 mg/L over the time of investigation.

Fluorimetric on-line measurements revealed that differences in concentrations of the tracer uranine in the entire free water body due mixing was <5% and was therefore considered complete mixed 5 h after dosing. Measured imidacloprid concentrations at end of each of the first 3 pulses ranged from 11.58 to 12.02 µg/L and from 11.12 to 11.42 µg/L in the 2nd series (Mohr et al., 2012). After flushing, concentrations were 0.08–0.1 µg/L, and the concentration of the tracer uranine was zero.

Prior to imidacloprid pulsing, benthic sampling yielded 48 taxa of all relevant feeding groups (for details see Mohr et al., 2012) with densities up to about 750 (spring) and 2500 (summer) gammarids per m$^2$ and about 250 insect larvae per m$^2$ in both spring and summer. There were characteristic habitat preferences of taxa for the 3 strata sampled (Fig. 3). While *Baetis* sp. was mostly found on the walls, *Caenis* sp. was mainly found in the straw. Tanypodinae and Chironomini also preferred the straw whereas the majority of the Orthocladiinae *Corynoneura* sp. stayed at the walls. The other Orthocladiinae and the Tanytarsini were present on all 3 strata with a slight preference for straw. In contrast, *G. roeseli* < 3.8 mm was most abundant on sand (Fig. 3).

Driftnet catches prior to imidacloprid pulsing revealed a significant pattern of higher night drift in *Baetis* sp. (night-time drift was higher than day-time drift in 23 measurements, it was lower in 4 measurements, and equal in 5 measurements, Fig. 4), chironomids (30–32 out of 32 catches) with the exception of Tanytarsini and
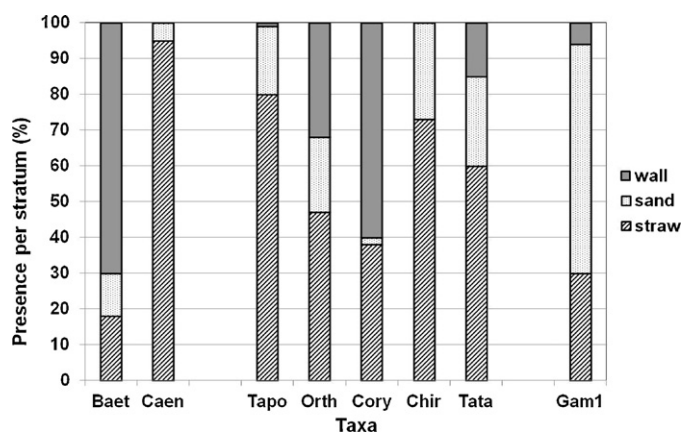


**Fig. 3.** Preference of taxa per stratum (%). Baet = *Baetis* sp., Caen = *Caenis* sp., Tapo = Tanypodinae, Orth = Orthocladiinae, Cory = *Corynoneura* sp., Chir = Chironomini, Tata = Tanytarsini, and Gam1 = *G. roeseli* < 3.8 mm.
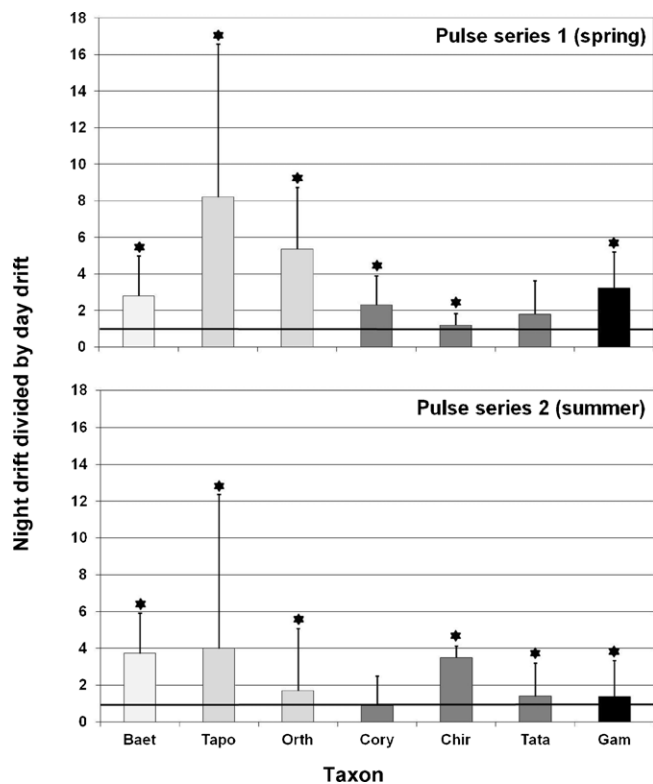
**Fig. 4.** Mean increase in night drift (factor) compared to day drift in the week prior to imidacloprid dosing of pulse series 1 (spring, 32 comparisons) and pulse series 2 (summer, 24 comparisons). Baet = *Baetis* sp., Caen = *Caenis* sp., Tapo = Tanypodinae, Orth = Orthocladiinae, Cory = *Corynoneura* sp., Chir = Chironomini, Tata = Tanytarsini, and Gam = *G. roeseli* (all size classes). The solid line at factor 1 indicates the level at which night drift equals day drift. Bars indicate standard deviation. Stars indicate significantly higher night drift (Wilcoxon signed-rank test, $p < 0.05$).

the Orthocladiinae *Corynoneura* sp., and *G. roeseli* (all 32 catches). Only a few specimens of *Caenis* sp. (Ephemeroptera) were caught in the driftnets even though this genus occurred in the mesocoms with up to 16 specimens per m$^2$ (Mohr et al., 2012). In the week before the 1st pulse series, 15 out of 32 night catches were higher than the corresponding day catch, in 16 cases night and day catches were equally zero. Drift net catches of Prodiamesinae (Chironomidae) were zero and occasionally one, reflecting the low abundance of this taxon in the mesocoms. Due to technical problems, the total number of catches for the comparison between day and night prior to the 2nd pulse series was reduced from 32 to 24. In *Baetis* sp. and Chironomidae the phenomenon of higher night drift became more pronounced in the 2nd pulse series in summer (Fig. 4). In the other taxa, this decrease compared to the spring series was less distinct. For *Caenis* sp. (data not shown), only 7 night catches out of 24 were higher than the day catches. Driftnet catches were almost identical in the 2 simultaneously exposed nets per stream. The same was true if 3 and 4 instead of 2 nets were exposed and the flow distance between nets was reduced from 20 to 10 m.

During the first imidacloprid pulse, drift of *Baetis* sp. increased by a factor of more than 6 (Fig. 5) and went almost down to zero after flushing with uncontaminated water. Similarly, increased drift in *Baetis* sp. could only be detected during the 3 pulses of the second series. In *Corynoneura* sp. (Orthocladiinae) drift increased during the 1st pulse up to a factor of 17.7 and was still high in the second night, when imidacloprid had already been flushed out for more than 12 h (Fig. 5). The other Orthocladiinae reacted with increased drift from the 2nd pulse on. The pattern, however, was different, since drift in the treated streams did not become higher than in the controls until the mesocoms had been flushed. In contrast,

Tanypodinae showed no increase in drift. Drift in *G. roeseli* > 3.8 mm total length was slightly higher than in the controls in both the first and second series and then decrease pulse by pulse below the control level (Fig. 5). In contrast, drift increased in *G. roeseli* < 3.8 mm by a factor of almost 19 and exceeded 37 on the following day, even though there was no more imidacloprid left in the water. In the second pulse series in June, the factor for *G. roeseli* < 3.8 mm was 5.3 during the first pulse and 9.3 on the following day. A pulse by pulse decrease in drift as in the other taxa was again evident.

The activity measurements with the MFB supported the drift net results. *B. fuscatus* exhibited a diurnal activity pattern with peaks around midnight and noon, which became more pronounced during and after imidacloprid exposure (Fig. 6). The activity was more or less the same in the controls whereas it decreased in the trials with the imidacloprid pulses. Spontaneous activity in *G. roeseli* was extremely high and was lower in the controls in the early morning (Fig. 7). During their 42 h stay in the test chambers, the activity of adult gammarids in the controls increased, presumably in search for food, whereas it decreased in the treatments. The decrease in activity and the time for recovery became higher with every pulse. Juvenile gammarids could not be used since activity decreased in the controls most likely due to the much higher food conversion rate and the resulting relative food demands compared to adult specimens.

## 4. Discussion

In this study, 12 h pulses of 12 μg/L imidacloprid provoked taxon-specific, immediate, and pronounced increases in the drift of insect larvae and *G. roeseli* < 3.8 mm and a pulse by pulse decrease in activity of exposed adult *G. roeseli*. Other factors, which are known to induce drift, such as drastic changes in water temperature, pronounced increase in current velocity, and rapid rise in turbidity (Allan, 1995), can be excluded as triggers since they remained constant before, during and after each pulse and an increase in drift intensity was not observed in the controls. The findings point out currently poorly regarded cumulative sublethal effects and incomplete recovery between pulses of imidacloprid set at short time intervals. In *G. roeseli*, drift occurred at concentrations, which were 2.5-times lower than the lowest concentration tested for *G. pulex* in the laboratory study of Beketov and Liess (2008a) and might occur at even lower imidacloprid concentrations. The results presented here do not exclude the possibility of even more pronounced effects on other species (e.g., drift in *B. rhodani* at 1 μg/L after 4 h, Beketov and Liess, 2008a) and developmental stages, or for other endpoints.

Among other things, imidacloprid is known to decrease feeding rates of mayflies at concentrations >1 μg/L (Alexander et al., 2007) and cause incomplete moulting of crustaceans (Song et al., 1997). Strong effects on mayfly instars were observed after a single 24 h imidacloprid pulse of 2.1 μg/L (Alexander et al., 2007). Moreover, at sublethal imidacloprid levels simultaneous exposure to other stressors like trout kairomones may result in ecologically relevant changes in behaviour as reported by Pestana et al. (2009) for the midge *C. riparius* and the caddisfly *Sericostoma vittarum*. Effects on growth and emergence of mayflies have also been found at very low sublethal concentrations (Alexander et al., 2008; Mohr et al., 2012).

In the following, the drift simulation quality of the study will be discussed first. Answers will be given to the key question, if the behaviour of the animals in the control mesocoms can be considered normal in regards to diurnal patterns of macroinvertebrate drift and natural drift intensity known from field observations. Thereafter both the distance travelled during drift events and drift intensity will be taken as measure to evaluate the ecological relevance of the repeated imidacloprid pulse effects.
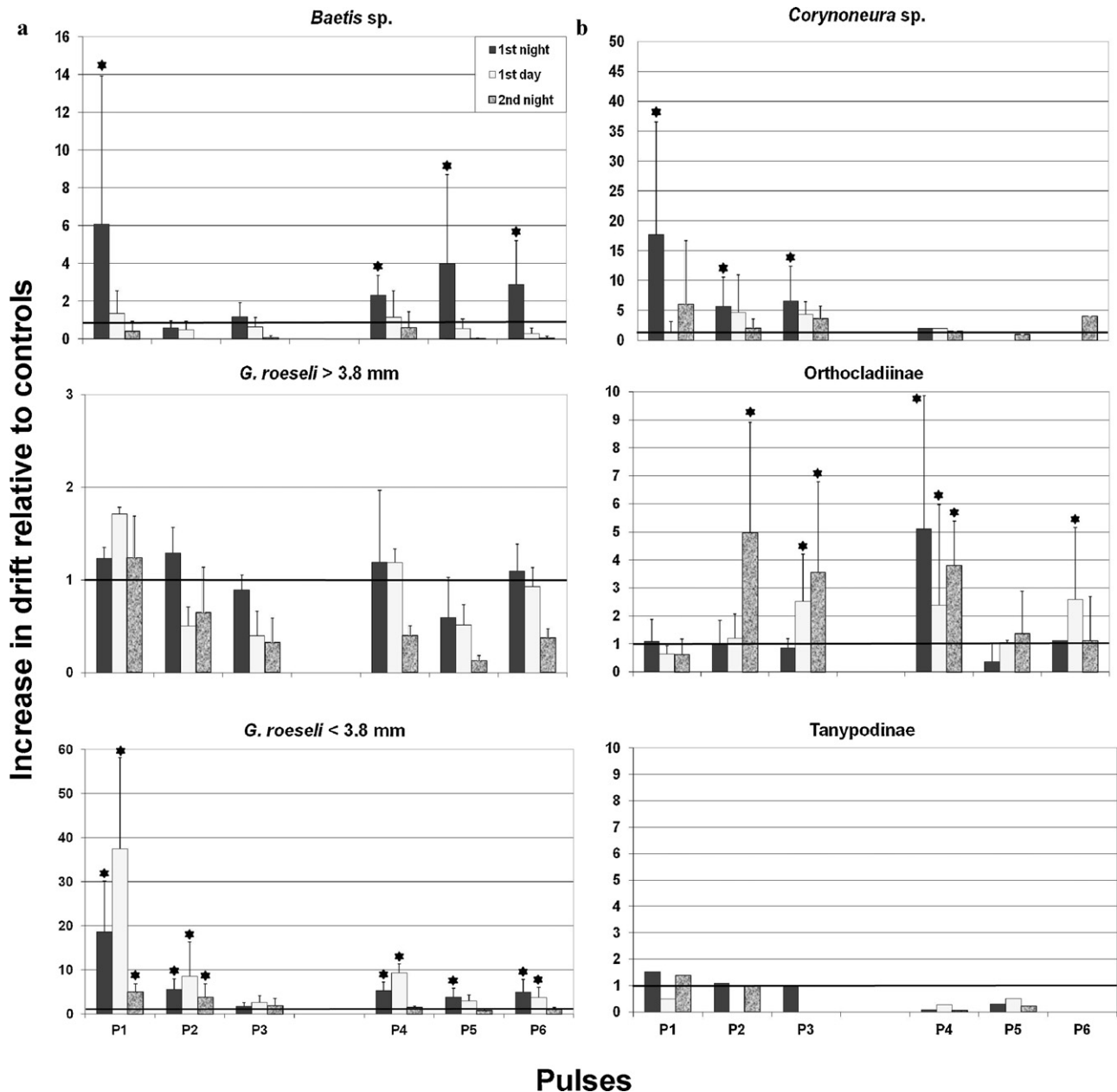
**Fig. 5.** (a) Increase in drift (factor) during and after the imidacloprid pulses (P1–P3 in spring and P4–P6 in summer) compared to the controls for *Baetis* sp., *G. roeseli* > 3.8 mm total length, and *G. roeseli* <3.8 mm total length, (b) for *Corynoneura* sp., other Orthocladiinae, and Tanypodinae. The solid line at factor 1 indicates the level at which imidacloprid drift equals control drift. Bars indicate standard deviation. Stars indicate significantly higher drift in treatments (Wilcoxon signed-rank test, $p < 0.05$).

Prior to the conclusions some information on imidacloprid contamination in the field will be given and implications for regulation will be addressed.

### 4.1. Drift simulation quality of the study

Regarding species composition and density, Mohr et al. (2012) already demonstrated that the mesocosm design used for this study meets the conditions in the reference stream fairly well. The diurnal pattern of increasing macroinvertebrate drift during the night, as detected in the artificial streams in the weeks prior to insecticide dosing and in the course of the spring and summer experiments, is a common phenomenon in running waters and may often amount to one order of magnitude (Allan, 1995). An important time signal (Zeitgeber) is light as already demonstrated for *Baetis* by Müller

(1954). There may also be differences in diurnal drift patterns at the species level. In the field, Lagrue et al. (2011) found adult *G. pulex* drifting at constant rates and co-occurring adult *G. roeseli* exhibiting a pattern of higher night drift compared to the day. Increased night drift as predator avoidance occurs in particular if vision-oriented predators like fish are abundant (cp. Smock, 2007), which was also the case in the reference stream. So in the artificial streams, the introduced organisms behaved as would have been expected in the reference stream even though there was no more fish present. The diurnal activity patterns (Allan, 1995) of *Baetis* and *G. roeseli* in the MFB were similar to those observed under natural conditions (Müller, 1954; Lagrue et al., 2011) providing support for the conclusion that the simulation of field conditions in the mesocosms was satisfactory with regard to animal behaviour.
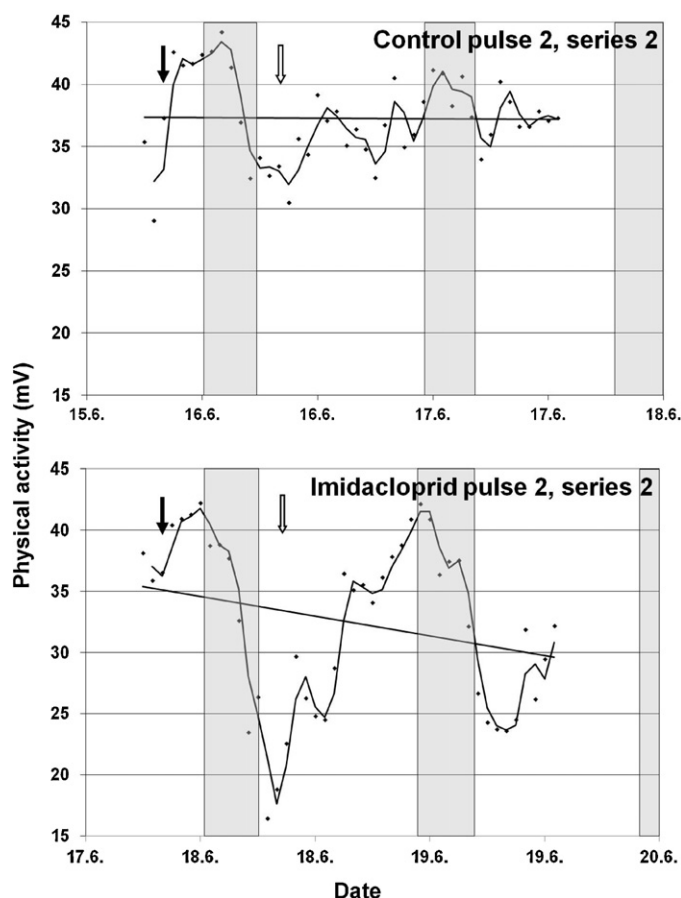
**Fig. 6.** Activity of *Baetis fuscatus* (mV) in the in situ exposed MFB test chambers in the control mesocosms (upper part) and the treatments (lower part). Black arrows indicate start of pulse, white arrows indicate end of pulse. Grey columns = complete darkness.

### 4.2. Drift intensity and the distance travelled under uncontaminated conditions

In many field experiments on macroinvertebrate drift, the share of drifters in the population was estimated on the basis of drift net catches and was in the range of 0.0–0.5% (Elliott, 1967; Ulfstrand, 1968; Bishop and Hynes, 1969). For comparison, population-related drift estimates were also calculated for the data generated in this study. As a result, the very low drift net catches in the stream mesocosms under uncontaminated conditions translated into a theoretical share of 0.05–0.2% for the gammarid, of 0.4–1.4% for the ephemerid and of 1–3% for the chironomid population. This order of magnitude is well in the range reported elsewhere. The drift intensity in the mesocosms can thereby be considered comparable to the field situation.

However, these population-related drift data have to be interpreted with caution since it may give the wrong impression that only a small part of the population exhibits downstream drift. In contrast, macroinvertebrate drift has to be looked at, at least under normal and low flow conditions, rather as a minor downstream shift of more or less the entire population, which is, of course, under normal conditions at least partly compensated by positive rheotaxis, downstream drift from upstream stretches and other mechanisms (see Elliott, 2002a,b,c). Indeed, drift distances of invertebrates were low in the controls as indicated by the almost identical catches of the 2 synchronously operated drift nets in each stream. This was even true when the number of nets was doubled and the distances between drift nets halved. As a result, the catches

of the drift nets were not related to the population size in further evaluation but as catches compared to the controls.

Under natural conditions, the drift distance is indeed in the range of only a few decimetres to metres in *B. rhodani* and *G. pulex* (Elliott, 1971; Townsend and Hildrew, 1976) and may generally vary during downstream drift with species, water velocity (Borchardt, 1993; Elliott, 2002b), and perhaps with turbulence (Wallace and Anderson, 1996). Lehmann (1967) studied drift and population dynamics of *G. pulex fossarum* Koch in the field employing electric fishing and radioisotope tagging. The mean distance travelled per day was about 2 m. Downstream movements were partly compensated for by positive rheotaxis. However, Waters (1965) estimated drift distances of up to 60 m. The time spent in the drift may vary considerably and depends on the taxon. Elliott (2002b) divided the drifters into three groups. Group 1 covers taxa like Chironomidae and Elmidae, which drift similar to dead invertebrates. Group 2 does not drift differently from dead invertebrates at water velocities >0.19 m/s and includes among other taxa the Simuliidae. The members of group 3 are represented by Ephemeroptera and Gammaridae. Drifters return to the stream bed more or less rapidly. In their study on drift distance Danehy et al. (2011) showed 'the majority of invertebrate drift' to be 'of local origin in headwater streams during summer baseflow.'

### 4.3. Increase in drift intensity in the presence of imidacloprid

According to both the fluorimetric measurements and the imidacloprid analyses before and after flushing with uncontaminated water, mixing in the water column of the streams was optimal (cp. Schulz et al., 2011) and the important targets of experimental conditions concerning pulse setting were achieved: (1) The real concentrations were very close to nominal concentrations of 12 µg/L imidacloprid. (2) The realistic application in the dark, which helped to minimize photolytic degradation, produced constant pulses as intended. (3) The imidacloprid concentrations after flushing the streams were very low and considered negligible with regard to their ecotoxicological potential (Mohr et al., 2012).

Drift numbers were highest in the presence of imidacloprid and decreased pulse by pulse in *Baetis* sp., *Corynoneura* sp., and *G. roeseli* < 3.8 mm. These taxa were most abundant on the exposed strata: the mesocosm walls and sand (Fig. 3), whereas on the days before the insecticide pulses the non-drifters *Caenis* sp. and Tanypodinae were mainly found in the straw. Unlike the vegetated areas in the stream mesocosm experiment by Beketov and Liess (2008b), the even distribution of the tracer uranine indicated the straw areas to have been as contaminated with imidacloprid as the circulating water. For that reason, the different hydraulics in the straw providing areas of reduced current velocity rather than lower imidacloprid concentrations may explain the absent downstream drift in the collector–gatherer *Caenis* sp. The genus *Caenis* is equipped with beating gills, which allow it to occur in 'slow weed-choked streams' (Whitney, 1939). In contrast, *Baetis* has motionless gills, which force these animals to a certain extent to seek the water current for oxygen supply rather than to avoid it. This is why the scraper and collector–gatherer *Baetis* (Elliott et al., 1988) is often found on stones in swift water (Whitney, 1939) and may explain its preference for the current-exposed mesocosm walls. Correspondingly, *Corynoneura* sp. is a free-ranging chironomid genus (Tokeshi, 1995) which makes it more prone to enter downstream drift in the case of insecticide pulses than Tanypodinae attaching with their prolegs to fallen leaves. Accordingly, Orthocladiinae and Tanytarsini which were abundant on all 3 strata, only showed an increase in drift after the 2nd pulse. Adult gammarids were even observed to seek shelter in the straw from the additional stressor of the water current and increasingly avoided the exposed sand areas (Mohr et al., 2012), which may result in a lower risk of predation under natural
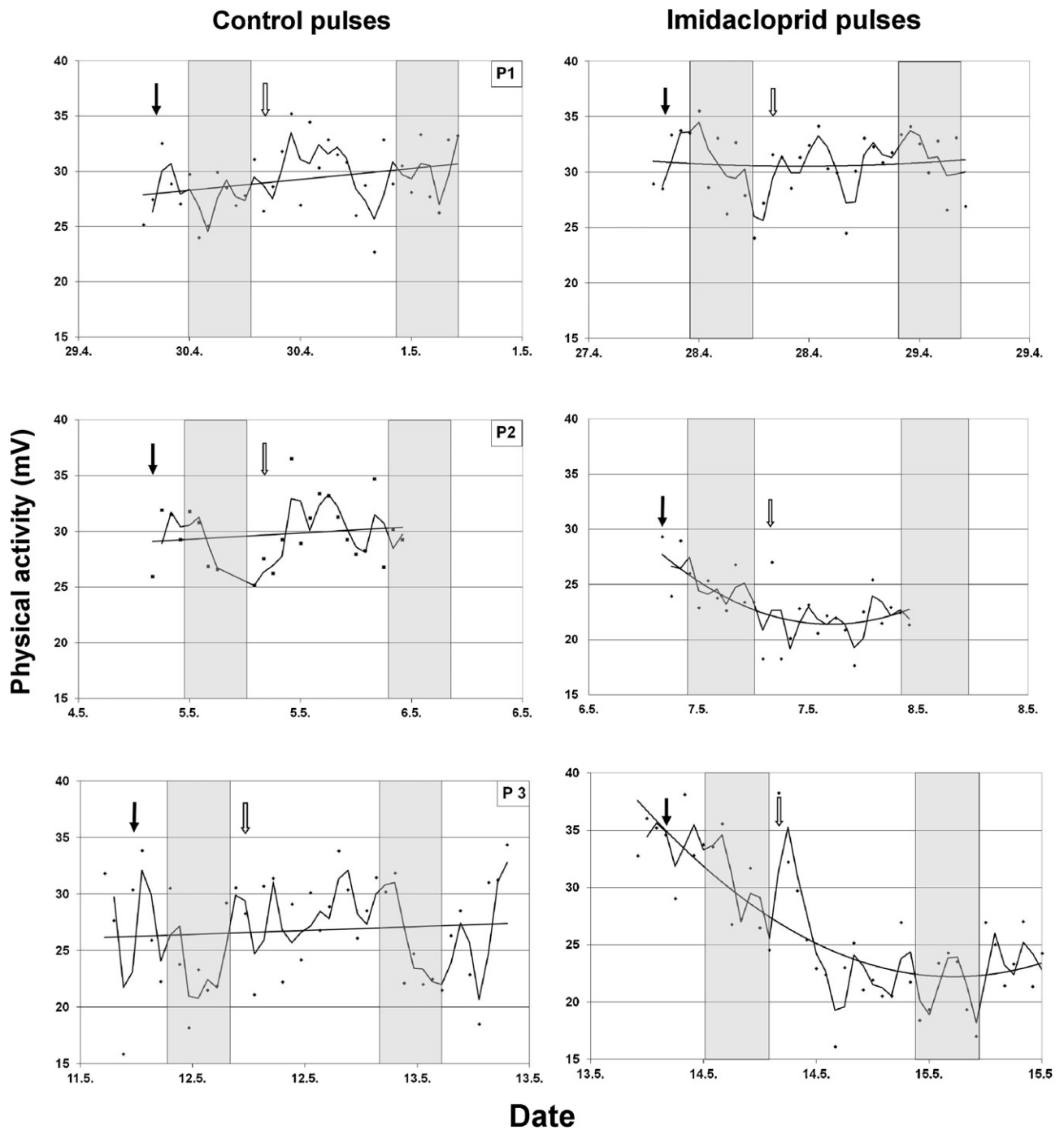
**Fig. 7.** Physical activity of *G. roeseli* (mV) in the in situ exposed MFB test chambers in the mesocosms. Controls of pulse 1–3 (left) and the corresponding treatments (right). Black arrows indicate start of pulse, white arrows indicate end of pulse. Grey columns = complete darkness.

conditions. This may also — besides a potential higher physiological sensitivity — explain why gammarids < 3.8 mm were much more affected by the imidacloprid pulses than the adults, since apart from their smaller size, the early juveniles were also much more abundant on sand as compared to straw (Fig. 3). This phenomenon became less pronounced in the course of the pulse series reflecting the growth of the specimens in the smallest size class.

### 4.4. Distance travelled during pesticide induced drift events

Besides drift intensity, the distance travelled is essential information for the evaluation of chemically induced drift events. In

this context, it is important to know if it is dealt with through active or passive entry into drift. Under normal conditions, Elliott (2002c) observed both active and passive entry into drift in *G. pulex* which had already been detected by Lancaster (1999) in *Ephemerella ignita* vs. the dytiscid beetle *Oreodytes sanmarkii*. As response to pyrethroid contamination, active entry into drift was observed by Lauridsen and Friberg (2005) in *Baetis*, gammarids, and stoneflies (*Leuctra*), with the latter even trying to avoid the pesticide lambda-cyhalothrin by temporarily crawling above the water line. The discrepancy between the rapid escape strategy for avoidance of pollution and the higher risk of predation from drift feeders has been discussed by Lauridsen and Friberg (2005) for *Gammarus*. In

an in-stream mesocosm experiment Heckmann and Friberg (2005) studied macroinvertebrate drift in the course of 30-min lambda-cyhalothrin pulses of 0.005–10 μg/L. The pyrethroid caused a rapid and strong drift increase in *G. pulex*, various Ephemeroptera, *Leuctra* sp., and Simuliidae, which was positively correlated with the tested concentrations. The community, however, was only affected at the highest concentrations of 5 and 10 μg/L and recovered within two weeks. In contrast, neonicotinoids like imidacloprid rather seem to promote passive entry into drift as indicated by the results of this study. The neurotoxicant seems to have reduced the capability of invertebrates to control displacement by the water current. In response, *G. roeseli* became less active and tried to avoid current stress by aggregating in areas like the straw (Mohr et al., 2012). In the early juveniles of this species, drift increased by a factor of 17.7 due to the toxicant. The increase was even more pronounced on the next day after flushing with uncontaminated water and was still to be seen in the following night. It became less pronounced from pulse to pulse. The juvenile stages were impacted more strongly, probably due to both their relatively higher and faster uptake of imidacloprid and their greater exposure to the water current. Nevertheless, under low current conditions as given in the mesocosm experiment, displacement was ecologically irrelevant. However, repeated pulses at short intervals, which do not allow the animals to detoxify the chemical (see Mohr et al., 2012), may have cumulative effects resulting in passive entry into drift without the possibility of regaining the sediment and thereby become ecologically relevant in view of the much greater distance travelled. According to Ashauer et al. (2010) the elimination time of imidacloprid in *G. pulex* amounted to 11.2 days after single 24 h exposure. In this species, the pesticide is evidently not metabolized (Ashauer et al., 2012), which may explain the findings for *G. roeseli* with the MFB in the study presented here. The more 12 h pulses were set at 7 days intervals the more pronounced was the decrease in motility of the animals accompanied by slower recovery, but there was no decrease in abundance (Mohr et al., 2012). Similar mechanisms of detoxification can be presumed for other species, in this case in particular insect larvae.

According to Williams and Hynes (1976) drift from upstream is the most important mechanism for recolonization. After physical disturbance, it can be rapid and considerable (Müller cit. Allan, 1995). However, positive rheotaxis and the resulting migration from downstream may also be relevant (Allan, 1995). Moreover, egg-laying by aerial adults (Smock, 2007) and transport by vectors like fish and waterfowl are important mechanisms of dispersal and colonization with the former being affected by flight activity and direction of the corresponding species, wind force and direction, time of the year and the distance from source areas. In this respect, plurivoltine taxa like chironomids are generally regarded less vulnerable, even though their range of dispersal by emerging adult can be considered much smaller compared, for example, to dragonflies, which have a long larval phase of up to 3 years, but a flight range of up to 30 km. In this study, re-colonization was simulated by restocking after the first series of pulses.

### 4.5. Relevance of imidacloprid pulses in the field

Insecticide pulses and corresponding effects are difficult to investigate in the field due to the small time windows of exposure. Höcker (2001) reported increased mortality, abnormal behaviour, and significantly reduced pupation from 11 to 15% down to only 1% of in situ caged trichopterans *Hydrospyche siltalai* in the small creek 'Schmiedbach', which flows through a hop growing region in Bavaria (Southern Germany) and discharges into the 'Siegbach'. In contrast, survival rates of 100% of in situ caged *G. roeseli*, was evident in this tributary compared to the 'Siegbach' and another tributary. In the 'Schmiedbach', the highest imidacloprid

concentrations were found with a mean of 0.1 μg/L and a maximum of 0.9 μg/L. Of course, these concentrations measured in the few samples taken in the course of the season cannot reflect the pulse regime, which the stream and its fauna may have been exposed to. Moreover, imidacloprid effects may have been masked in the main stream and the second tributary by adsorption to seston or hydraulic retention in the hyporheic zone (cp. Mutz et al., 2007). These assumptions were supported by high imidacloprid concentrations in the sediment of 344 μg/kg dry weight in August, which is the time right before the hop harvest, when imidacloprid is no longer applied. Höcker also found that there was no correlation between the organic content of the seston and its imidacloprid concentration and that it was easy to elute the insecticide. In any case, this study illustrates the ecological relevance and the complexity of the pulse contamination issue.

### 4.6. Implications for regulation

At the low water velocity given in this study, the neurotoxic effect of imidacloprid induced a pronounced increase in passive entry into drift. In a laboratory study with *G. pulex* (L.), the endpoint drift responded after only 2 h at the lowest concentration tested which was 10 times lower than the $LC_{50}$ after 96 h (Beketov and Liess, 2008a). Lethal levels can definitely be much lower in more sensitive species like Ephemeroptera (Beketov and Liess, 2008a) and may be also lower in *G. roeseli*. The endpoint drift is related to possible ecological implications, since drifting invertebrates may become more accessible to predators (Allan, 1995; Brooks et al., 2009) and might, in the case of long distance travel, even drift out of the catchment area. Moreover, besides repeated pulses, the interaction between pesticide and other chemicals present in bodies of water, which repel rather than kill freshwater invertebrates (cp. Klaschka, 2009) must be regarded, too.

Consequently, drift is a sensitive endpoint of regulatory relevance. The carrousel drift meter of Werth and Marten (2007), the artificial stream used by Beketov and Liess (2008a), and video tracking methods of Nørum et al. (2010) can be regarded promising tools in rapid screening of these substances for potential drift initiating effects. In any case, the potential of a chemical to induce drift should be regarded when a specific risk assessment for lotic surface waters is done, i.e. in the context of a spatially explicit risk assessment. If drift cannot be measured (i.e. in a lotic model ecosystem or with a drift meter), the remaining uncertainty regarding the relative sensitivity of the endpoint drift could also be addressed by applying a suitable safety factor.

### 4.7. Conclusions

Repeated short-term pulses of imidacloprid affected the stream fauna even in assumed retreat areas. Macrozoobenthos drift should be considered as a relevant endpoint in risk assessments. The environmental impact of chemicals on drift is, besides factors like water current and water temperature, not only a function of pulse height and pulse duration, but most importantly also of the time interval between pulses and the possibility for the animals to detoxify, which is species and size specific.

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## References

Alexander, A.C., Culp, J.M., Liber, K., Cessna, A.J., 2007. Effects of insecticide exposure on feeding inhibition in mayflies and oligochaetes. Environmental Toxicology and Chemistry 26, 1726–1732.

Alexander, A.C., Heard, K.S., Culp, J.M., 2008. Emergent body size of mayfly survivors. Freshwater Biology 53, 171–180.

Allan, J.D., 1995. Stream Ecology: Structure and Function of Running Waters. Chapman and Hall, London, pp. 221–377 (Chapter 10: Drift).

Ashauer, R., Caravatti, I., Hintermeister, A., Escher, B.I., 2010. Bioaccumulation kinetics of organic xenobiotic pollutants in the freshwater invertebrate *Gammarus pulex* modelled with prediction intervals. Environmental Toxicology and Chemistry 29, 1–12.

Ashauer, R., Hintermeister, A., O'Connor, I., Elumelu, M., Hollender, J., Escher, B., 2012. Significance of xenobiotic metabolism for bioaccumulation kinetics of organic chemicals in *Gammarus pulex*. Environmental Science & Technology, http://dx.doi.org/10.1021/es204611h.

Beketov, M.A., Liess, M., 2008a. Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates. Archives of Environmental Contamination and Toxicology 55, 247–253.

Beketov, M.A., Liess, M., 2008b. Variability of pesticide exposure in a stream mesocosm system: macrophyte-dominated vs. non-vegetated sections. Environmental Pollution 156, 1364–1367.

Bendschneider, K., Robinson, R.J., 1952. A new spectrophotometric method for the determination of nitrite in sea water. Journal of Marine Research 11, 87–96.

Bishop, J.E., Hynes, H.B.N., 1969. Downstream drift of the invertebrate fauna in a stream ecosystem. Archiv für Hydrobiologie 66, 56–90.

Borchardt, D., 1993. Effects of flow and refugia on drift loss of benthic macroinvertebrates: implications for habitat restoration in lowland streams. Freshwater Biology 29, 221–227.

Breneman, D.H., Pontasch, K.W., 1994. Stream microcosm toxicity tests: predicting the effects of fenvalerate on riffle insect communities. Environmental Toxicology and Chemistry 13, 381–387.

Brooks, A.C., Gaskell, P.N., Maltby, L.L., 2009. Sublethal effects and predator–prey interactions: implications for ecological risk assessment. Environmental Toxicology and Chemistry 28, 2449–2457.

Burdick, G.E., Dean, H.J., Harris, E.J., 1960. Effect of sevin upon the aquatic environment. New York Fish and Game Journal 7, 14–25.

Canadian Council of Ministers of the Environment (CCME), 2007. Canadian Water Quality Guidelines: Imidacloprid Scientific Supporting Document. Canadian Council of Ministers of the Environment, Winnipeg, PN 1388.

Chaney, A.L., Marbach, E.P., 1962. Modified reagents for determination of urea and ammonia. Clinical Chemistry 8, 130–132.

Danehy, R.J., Langshaw, R.B., Duke, S.D., Bilby, R.E., 2011. Drift distance of macroinvertebrates throughout summer in headwater tributaries of the Calapooia River. Fundamental and Applied Limnology 178 (2), 111–120.

DIN 38405, 1990. Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung; Anionen (Gruppe D). Photometrisches Verfahren von gelöster Kieselsäure. Part 21, D21.

DIN EN ISO 9963-1, 1996. Wasserbeschaffenheit. Bestimmung der Alkalinität. Teil. Bestimmung der gesamten und der zusammengesetzten Alkalinität.

DIN EN ISO 13395, 1996. Wasserbeschaffenheit – Bestimmung von Nitritstickstoff, Nitratstickstoff und der Summe von beiden mit der Fließanalytik (CFA und FIA) und spektrometrischer Detektion.

DIN EN ISO 15681-1, 2004. Wasserbeschaffenheit – Bestimmung von Orthophosphat und Gesamtphosphor mittels Fließanalytik (FIA und CFA) – Teil 1: Verfahren mittels Fließinjektionsanalyse (FIA).

DIN EN ISO 11732, 2005. Wasserbeschaffenheit – Bestimmung von Ammoniumstickstoff – Verfahren mittels Fließanalytik (CFA und FIA) und spektrometrischer Detektion.

Dosdall, L.M., Lehmkuhl, D.M., 1989. Drift of aquatic insects following methoxychlor treatment of the Saskatchewan river system, Canada. Canadian Entomologist 121, 1077–1096.

Elliott, J.M., 1967. Invertebrate drift in a Dartmoor stream. Archiv für Hydrobiologie 63, 2022–2237.

Elliott, J.M., 1971. The distances travelled by drifting invertebrates in a Lake District stream. Oecologia 6, 191–220.

Elliott, J.M., Humpesch, U.H., Macan, T.T., 1988. Larvae of the British Ephemeroptera: A Key with Ecological Notes. Wilson, Kendal.

Elliott, J.M., 2002a. A continuous study of the total drift of freshwater shrimps, *Gammarus pulex*, in a small stony stream in the English Lake District. Freshwater Biology 47, 75–86.

Elliott, J.M., 2002b. The drift distances and time spent in the drift by freshwater shrimps, *Gammarus pulex*, in a small stony stream, and their implications for the interpretation of downstream dispersal. Freshwater Biology 47, 1403–1417.

Elliott, J.M., 2002c. Time spent in the drift by downstream-dispersing invertebrates in a Lake District stream. Freshwater Biology 47, 97–106.

FOCUS, 2001. FOCUS Working Group on Surface Waters. FOCUS surface water scenarios in the EU evaluation process under 91/414/EEC; EC document SANCO/4802/2001-rev.2 final. Brussels.

Gerhardt, A., Carlsson, A., Ressemann, C., Stich, K.P., 1998. A new online biomonitoring system for *Gammarus pulex* (L) (Crustacea): *in situ* test below a copper effluent in south Sweden. Environmental Science & Technology 32, 150–156.

Hall, R.J., Likens, G.E., Fiance, S.B., Hendrey, G.R., 1980. Experimental acidification of a stream in the hubbard brook experimental forest New-Hampshire USA. Ecology (Washington, DC) 61, 976–989.

Heckmann, L.-H., Friberg, N., 2005. Macroinvertebrate community response to pulse exposure with the insecticide lambda-cyhalothrin using in-stream mesocosms. Environmental Toxicology and Chemistry 24, 582–590.

Hensel, S., Kiel, E., 2006. Zur Simulation eines Tieflandbaches in Fließgewässer-Mesokosmen – Konzeption und erste Ergebnisse. Tagungsberichte der Deutschen Gesellschaft für Limnologie. Werder, 450–454.

Höcker, B., 2001. Biozönotische Typisierung und ökotoxikologische Untersuchungen an zwei kleinen bayerischen Fließgewässern. Bayerisches Landesamt für Wasserwirtschaft, Materialien 96, 192 pp. (appendix).

Jemec, A., Tisler, T., Drobne, D., Sepcic, K., Fournier, D., Trebse, P., 2007. Comparative toxicity of imidacloprid, of its commercial liquid formulation and of diazion to a non-target arthropod, the microcrustacean *Daphnia magna*. Chemosphere 68, 1408–1418.

Kingsbury, P.D., 1976. Studies of the aerial applications of the synthetic pyrethroid NRDC-143 on aquatic ecosystems. Chemical Control Research Institute Information Report CC-X-127, Ottawa, Canada, 111 pp.

Klaschka, U., 2009. A new challenge – development of test systems for the infochemical effect. Environmental Science and Pollution Research International 16, 370–388.

Lagrue, C., Kaldonski, N., Motreuil, S., Lefèvre, T., Blatter, O., Giraud, P., Bollache, L., 2011. Interspecific differences in drift behavior between the native *Gammarus pulex* and the exotic *Gammarus roeseli* and possible implications for the invader's success. Biological Invasions 13, 1409–1421.

Lancaster, J., 1999. Small-scale movements of lotic macroinvertebrates with variations in flow. Freshwater Biology 41, 605–619.

Lauridsen, R.B., Friberg, N., 2005. Stream macroinvertebrate drift response to pulsed exposure of the synthetic pyrethroid lambda-cyhalothrin. Environmental Toxicology 5, 513–521.

Lehmann, J., 1967. Drift und Populationsdynamik von Gammarus pulex fossarum Koch. Zeitschrift für Morphologie und Ökologie der Tiere 60, 227–274.

Liess, M., 1994. Pesticide impact on macroinvertebrate communities of running waters in agricultural systems. Verhandlungen der Internationalen Vereinigung für Limnologie 25, 2060–2062.

Lozan, J.L., Kausch, H., 1998. Angewandte Statistik für Naturwissenschaftler, second ed. Parey, Berlin.

MacDonald, L.M., Meyer, T.R., 1998. Determination of imidacloprid and triadimefon in white pine by gas chromatography/mass spectrometry. Journal of Agricultural and Food Chemistry 46, 3133–3138.

Mohr, S., Feibicke, M., Ottenströer, T., Meinecke, S., Berghahn, R., Schmidt, R., 2005. Enhanced experimental flexibility and control in ecotoxicological mesocosm experiments – a new outdoor and indoor pond and stream system. Environmental Science and Pollution Research International 12, 5–7.

Mohr, S., Berghahn, R., Schmiediche, R., Hübner, V., Feibicke, M., Loth, S., Feibicke, M., Mailahn, W., Wogram, J., 2012. Macroinvertebrate community response to repeated short-term insecticide pulses. Aquatic Toxicology 110–111, 25–36.

Müller, K., 1954. Die Drift in fließenden Gewässern. Archiv für Hydrobiologie 49, 539–545.

Mullin, J.B., Riley, J.P., 1955. The colorimetric determination of silicate with special reference to sea and natural waters. Analytica Chimica Acta 12, 162–176.

Murphy, J., Riley, J.P., 1962. A modified method for the determination of phosphate in natural waters. Analytica Chimica Acta 27, 31–36.

Mutz, M., Kalbus, E., Meinecke, S., 2007. Effect of instream wood on vertical water flux in low-energy sand bed flume experiments. Water Resources Research 43, W10424, http://dx.doi.org/10.1029/2006WR005676.

Nørum, U., Friberg, N., Jensen, M.R., Pedersen, J.M., Bjerregaard, P., 2010. Behavioural changes in three species of freshwater macroinvertebrates exposed to the pyrethroid lambda-cyhalothrin: laboratory and stream microcosm studies. Aquatic Toxicology 98, 328–335.

Pegel, M., 1980. Methoden zur Erfassung der Abundanzdynamik der präimaginalen Stadien von Simuliiden (Diptera) – Entnahme quantitativer Proben mit Hilfe künstlicher Substrate. Diss. Univ. Hamburg, 175 pp.

Perkin, E.K., Hölker, F., Richardson, J.S., Sadler, J.P., Wolter, C., Tockner, K., 2011. The Influence of Artificial Light on Stream and Riparian Ecosystems: Questions, Challenges, and Perspectives., http://www.esajournals.org/doi/pdf/10.1890/ES11-00241.1.

Pestana, J.L.T., Loureiro, S., Baird, D.J., Soares, A.M.V.M., 2009. Fear and loathing in the benthos: responses of aquatic insect larvae to the pesticide imidacloprid in the presence of chemical signals of predation risk. Aquatic Toxicology 93, 138–149.

Schulz, M., Priegnitz, J., Klasmeier, J., Heller, S., Meinecke, S., Feibicke, M., 2011. Effect of bed surface roughness on longitudinal dispersion in artificial open channels. Hydrological Processes, http://dx.doi.org/10.1002/hyp.8229.

Schulz, R., Liess, M., 1999. A field study on the effects of agriculturally derived insecticide input on stream macroinvertebrate dynamics. Aquatic Toxicology 46, 155–176.

Schulz, R., 2008. Field studies on exposure, effects, and risk mitigation of aquatic non-point source insecticide pollution: a review. Journal of Environmental Quality 33, 419–448.

Sebastien, R.J., Brust, R.A., Rosenberg, D.M., 1989. Impact of methoxychlor on selected nontarget organisms in a riffle of the Souris river, Manitoba, Canada. Canadian Journal of Fisheries and Aquatic Sciences 46, 1047–1061.

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. Environmental Pollution 70, 35–55.

Sigg, L., Stumm, W., 1989. Aquatische Chemie: Eine Einführung in die Chemie wässriger Lösungen und natürlicher Gewässer. 3. Aufl. VDL, Zürich.

Smock, L.A., 2007. Macroinvertebrate dispersal. In: Hauer, F.R., Lamberti, G.A. (Eds.), Methods in Stream Ecology. Elsevier, Amsterdam, pp. 465–487.

Song, M.Y., Stark, J.D., Brown, J.J., 1997. Comparative toxicity of four insecticides, including Imidacloprid and Tebufenozide, to four aquatic arthropods. Environmental Toxicology and Chemistry 16, 2494–2500.

Svedsen, C.R., Quinn, T., Kolbe, D., 2004. Review of macroinvertebrate drift in lotic ecosystems – final report for Wildlife Research Program. Environment and Safety Division, Seattle City Light (manuscript), 92 pp.

Tennekes, H.A., 2010. Contamination of Dutch Surface Water with Imidacloprid Threatens Insects (last accessed July 2011) http://www.farmlandbirds.net/en/content/contamination-dutch-surface-water-imidacloprid-threatens-insects.

Tokeshi, M., 1995. Species interactions and community structure. In: Armitage, P.D., Cranston, P.S., Pinder, L.C.V. (Eds.), The Chironomidae: Biology and Ecology of Non-biting Midges. Chapman & Hall, London, pp. 297–335.

Townsend, C.R., Hildrew, A.G., 1976. Field experiments on the drifting, colonization, and continuous redistribution of stream benthos. The Journal of Animal Ecology 45, 759–772.

Ulfstrand, S., 1968. Benthic animal communities in Lapland streams. Oikos (Suppl. 10), 1–120.

Wallace, R.R., Hynes, H.B.N., 1975. The catastrophic drift of stream insects after treatments with methoxychlor (1,1,1-trichloro-2-2-bis (alpha-methoxyphenyl) ethane). Environmental Pollution 8, 255–268.

Wallace, J.B., Lugthart, G.J., Cuffney, T.F., Schnurr, G.A., 1989. Impact of repeated insecticidal treatments on drift and benthos of a headwater stream. Hydrobiologia 179, 135–147.

Wallace, J.B., Huryn, A.D., Lugthart, G.J., 1991a. Colonization of a headwater stream during 3 years of seasonal insecticidal applications. Hydrobiologia 211, 65–76.

Wallace, J.B., Cuffney, T.F., Webster, J.R., Lugthart, G.J., Chung, K., Goldowitz, B.S., 1991b. Export of fine organic particles from headwater streams: Effects of season, extreme discharges, and invertebrate manipulation. Limnology and Oceanography 36, 670–682.

Wallace, B.J., Anderson, N.H., 1996. Habitat, life history, and behavioural adaptations of aquatic insects. In: Merrit, R.W., Cummins, K.W. (Eds.), An Introduction to the Aquatic Insects of North America. Kendall/Hunt, Dubuque, pp. 41–73.

Waters, T.F., 1965. Interpretation of invertebrate drift in streams. Ecology 46, 327–334.

Werth, C., Marten, M., 2007. Arthropoden-Drift als neue sensitive Messgröße in der Echtzeitüberwachung von Fließgewässern mit kontinuierlichen Biotestverfahren. Deutsche Gesellschaft für Limnologie (DGL) – Tagungsbericht der Jahrestagung in Dresden 2006, Dresden, pp. 564–568.

Whitney, R.J., 1939. The thermal resistance of mayfly nymphs from ponds and streams. The Journal of Experimental Biology 16, 374–385.

Williams, D.D., Hynes, H.B.N., 1976. The recolonization mechanism of stream benthos. Oikos 27, 265–272.

Wood, E.D., Armstrong, F.A.J., Richards, F.A., 1967. Determination of nitrate in sea water by cadmium–copper reduction to nitrite. Journal of the Marine Biological Association of the United Kingdom 47, 23–31.

Yasuno, M.S., Fukushima, S., Hasegawa, J., Shioyama, F., Hatakeyama, S., 1982. Change in the benthic fauna and flora after application of temephos to a stream on Mt. Tsukuba. Hydrobiologia 89, 205–214.

Zwick, P., 1992. Fließgewässergefährdung durch Insektizide. Naturwissenschaften 79, 437–442.