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Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: Effect concentrations and recovery dynamics

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ABSTRACT

In pesticide risk assessment, effect concentrations and dynamics of long-term community-level effects caused by pulse exposures remain to be investigated. This is because long-term experiments are exceptionally rare, and most of the previously investigated communities had low proportions of sensitive long-living species. The aim of the present study was to investigate the effect of a single pulse contamination with the insecticide thiacloprid on invertebrates. We employed mesocosms designed to realistically mimic communities in small streams within the agricultural landscape. Specifically, the objectives were to (i) compare the community Lowest-Observed-Effect Concentration (LOEC) with organism-level median lethal concentrations (LC50), and (ii) to assess recovery dynamics with special focus on short- and long-living taxa. The contamination resulted in long-term alteration of the overall invertebrate community structure (7 months, until the end of the experiment). Long-term community LOEC was 3.2 μg/L (Redundancy Analysis), slightly below the acute LC50s known for sensitive invertebrates relevant to the mesocosm community. However, one species (stonefly *Nemoura cinerea*) was affected at the lowest tested concentration, 70 times below the lowest known LC50. Concerning time to recovery from the effect, we found that the duration depends on the life-cycle characteristics of species, but not on the toxicant concentration: short-living (mulivoltine) species recovered after 10 weeks following contamination, whereas long-living (uni- and semivoltine) species did not recover until the end of the experiment (7 months). The present example shows that concentrations of pesticides at which majority of the species is affected can be predicted by acute organism-level toxicity tests with sensitive species. However, tests with longer observation periods, as well as consideration of environmental factors and inter-taxon variability in sensitivity are required to predict effects on all species comprising a community. Realistic prediction of community recovery dynamics requires consideration of the species' life-cycle traits.

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1. Introduction

One of the crucial aims of ecotoxicology is to assess and define the concentration levels at which contaminants cause effects on communities and ecosystems, and to investigate and predict recovery of these systems following toxicant stress (e.g. Campbell et al., 1999; Giddings et al., 2002). Among the contaminants in current use, modern non-persistent insecticides as well as other pesticides are relevant stressors for many aquatic and terrestrial organisms (Liess et al., 2005), and a great number and variety of studies have been conducted to derive and predict the effect concentrations for these toxicants and to understand the processes of recovery from the effects of these contaminants.

Concentration levels for ecological effects of pesticides and many other toxicants are typically derived from laboratory single-species tests. Results of such tests are used for predicting potential effects of toxicants on ecosystems either by applying safety factors (e.g. EEC, 1991) or using species-sensitivity distribution (SSD) methods (e.g. Posthuma et al., 2002). In addition to these predictive methods based on laboratory single-species tests, a wide array of more complex experimental systems is used for validation of the laboratory tests in semi-natural conditions. These model ecosystems, referred to as micro- and mesocosms, are used for risk assessment of pesticides and are known as higher-tier risk assessment testing systems (Campbell et al., 1999).

For pesticides, a recent review focused on comparison of the result from laboratory and mesocosm test systems revealed that effects of these toxicants on biological communities in mesocosms have rarely been observed at concentrations >10 times lower than the acute Median Effective Concentrations (EC50) obtained for the species known to be sensitive in laboratory conditions (*Daphnia magna*), and in most cases have been observed at much higher concentrations (Van Wijngaarden et al., 2005). On the other hand, several microcosm studies focused on chronic post-exposure effects of insecticides have shown that these toxicants can have a long-term influence on most sensitive endpoints even at concentrations up to 1000 times lower than the laboratory-generated acute EC50s (for *D. magna* or sensitive insect species) (Lozano et al., 1992; Liess and Schulz, 1996; Liess, 2002; Beketov and Liess, 2005). In addition, existing field monitoring studies indicate that pesticides may have adverse effects on freshwater invertebrates at concentrations more than 100 times below the laboratory-generated acute EC50s derived for *D. magna* (Liess and von der Ohe 2005; Schäfer et al., 2007).

Recovery of ecological systems after chemical stress caused by pesticides and other environmental toxicants currently receives increasing attention from scientists and regulators (Giddings et al., 2002; Barnthouse 2004; Caquet et al., 2007). Investigations of the recovery processes usually employ micro- and mesocosms. For pesticides, community recovery in mesocosms is frequently observed within a relatively short period after contamination. Thus, for non-persistent insecticides the majority of previously published studies have shown that recovery is already completed within two months after contamination (reviewed by Van Wijngaarden et al., 2005). However, a few long-term mesocosm experiments have revealed that even a single short-term exposure to pesticides may result in long-term and permanent elimination of long-living species if external recolonisation is hampered (Van den Brink et al., 1996; Caquet et al., 2007). Hence, the rapid recovery observed in many mesocosm systems that are predominantly inhabited by short-living organisms (e.g. plankton and short-living benthic insects) and open for external recolonisation (e.g. aerial entry of insects from neighbouring controls) may easily underestimate the recovery duration for communities that include long-living species and are relatively isolated from unimpaired ecosystems (Caquet et al., 2007; Hanson et al., 2007).

Thus for pesticides uncertainty remains regarding both effect concentrations and recovery patterns. In the authors' opinion one main reason for this uncertainty is the paucity of long-term mesocosm experiments employing ecologically realistic communities with a large proportion of long-living taxa and extensive field monitoring studies. Long-term experimental studies are particularly important for understanding effects on long-living species, as experimental observation periods covering significant part of species life-spans are needed to understand duration of effects and recovery patterns (e.g. for univoltine taxa desirable observation period is from >0.5 year to ≤1 year).

Long-term mesocosm experiments are rare. To the authors' knowledge only 5 out of 62 community-level studies on non-persistent insecticides published so far (70 papers) include post-contamination observation periods longer than half a year. These are studies by Brock et al. (1992), Fairchild and Eidt (1993), Van den Brink et al. (1996), Woin (1998), and Hanson et al. (2007) (for the studies reported as paper series, only the first papers are cited). All these investigations were performed with standing-water systems.

Although these long-term studies were not focused on understanding the importance of species' life-cycle traits for post-exposure recovery, two of them have shown that recovery of long-living (univoltine) species after pronounced toxic effect can take long time periods comparable to the species' lifespans (≥1 year) (Van den Brink et al., 1996; Woin 1998). However, the numerical proportion of the long-living species (with generation time ≥1 year) in the communities analysed in these two studies was low (about 10 and 24% of the analysed communities respectively; own calculations based on reported information). Besides, long-term effects on the entire community structure were either not found under ecologically realistic conditions (as stated by the authors) because relatively few long-living taxa were affected (Van den Brink et al., 1996) or this aspect was not analysed (Woin, 1998). Importantly, invertebrate communities in natural streams uncontaminated with pesticides usually include much greater proportions of long-living taxa. For example in Europe, the percentage of the taxa having generation time ≥1 year in uncontaminated streams in France and Finland varies from 60 to 80% and from 40 to 70% of the overall taxa richness respectively (own calculations with data from Schäfer et al., 2007). However, significance and patterns of the long-term effects caused by single pulse contamination with an insecticide remain to be investigated.

The aim of the present study was to investigate long-term effects of a single pulse contamination with the neonicotinoid insecticide thiacloprid on invertebrate communities of stream mesocosms, which were allowed to establish a community

having a relatively high proportion of long-living univoltine taxa (about 50% of the taxa richness at levels of taxonomic identification similar to those used by Schäfer et al. (2007)) for 16 months before contamination. In particular, the objectives were (i) to derive the community Lowest-Observed-Effect Concentration (LOEC) and compare it with laboratory-generated toxicity data, and (ii) to assess the long-term effect-and-recovery dynamics with special focus on short- and long-living taxa. The insecticide was applied as a single pulse to simulate contamination due to spray drift or surface water runoff, which represent a relevant input path for small streams in agricultural areas (Liess et al., 1999, Neumann et al., 2002).

2. Materials and methods

2.1. *Description of artificial stream system*

The mesocosm system used in the present study consisted of 16 artificial streams. Each stream has the following characteristics: length 20 m, width at water surface 0.32 m (±0.03), average depth 0.25 m (±0.11), discharge 160 L/min (±9), slope 2%; approximate total volume 1000 L (range in parentheses). Each stream is designed as a closed circulation system. In this system the water flows as follows: from the upstream to the downstream sections of the stream it is propelled by gravity, then it falls down into the 200-L reservoir installed below the downstream margin of the stream, and then it is pumped back to the upstream section through a plastic tube (40 mm in diameter) by an electric pump (260W, Atlantis 150, OASE, Hörstel, Germany). At the end of each stream a dam with polyester net filter (1 mm mesh) is installed to prevent loss of the animals to the 200-L reservoirs.

The stream channels are situated in the ground to a depth of about 0.4 m, and lined with water-tight nontoxic polyvinylchloride foil (0.8 mm, Czebra, Lauterecke, Germany) to prevent leakage of water to the surrounding environment. The bottom of the streams is covered with a mixture of fine gravel and sand (particle size 0.2–3.7 mm, layer of 30–50 mm). The streams are located as parallel lines with 0.8 m distance between the stream channels on the territory of UFZ—Helmholtz Centre for Environmental Research (Leipzig, Germany). An area of ground was retained between the streams to support riparian vegetation, so as to provide a refuge for emerged insects, reduce the amount of direct sunlight, and in general to create as much ecological realism as possible.

The system was constructed in summer 2003. In September 2003 and April 2004—two years before contamination—the streams were planted with watercress *Nasturtium officinale*. In order to introduce macroinvertebrates into the streams the sediments (sand, clay, and organic debris) collected with a surber sampler (500 μm mesh) in an uncontaminated small stream near Gross Bardau village (south of Grimma city, Eastern Germany, 51°10′56 N and 12°46′29 E) were added to the streams. The sediments and in addition macroinvertebrates were added several times during winter 2004–2005, and also in October 2005 in order to mimic natural influx of species by drift.

The main physico-chemical parameters of water were measured approximately every four months starting from June 2005 (Table 1). Concentrations of ammonium, nitrite, nitrate, phosphate, and total hardness were determined with Aquamerck colorimetric tests (Merck, Darmstadt, Germany). Conductivity, dissolved oxygen, and pH were measured with LF330, OXI 340, and Multi 340i electronic meters respectively (WTW, Weilheim, Germany). No significant differences between the groups of treatment and control streams were found concerning the physico-chemical parameters ($P>0.05$, analysed for every measuring date with multivariate analysis of variance (MANOVA)).

Table 1 – Main physico-chemical parameters of water in stream mesocosms

Parameter	Mean	Standard deviation
Ammonium (mg/L)	0.053	0.11
Nitrate (mg/L)	4.04	6.4
Nitrite (mg/L)	0.004	0.01
Phosphate (mg/L)	0.21	0.15
Hardness (mg Ca/L)	57.14	2.44
Dissolved oxygen (%)	95.5	15.4
pH	7.88	0.17
Conductivity (μS/cm)	496.21	85.27

Temperature was measured constantly (every 3 h) by μS-LOG540 data logger (Driessen + Kern, Bad Bramstedt, Germany) in two randomly selected streams. The maximum summer (April to September) and minimum winter (October to March) temperatures were 25.9 and 2.7 °C respectively. Mean summer and winter temperatures were 20.33 and 4.52 °C respectively. To compare temperature regimes in all streams DK501-PL data loggers (Driesen + Kern, Bad Bramstedt, Germany) were located in each stream for one month (02–30.03.2007) to measure temperature every 3 h. No significant differences between the groups of treatment and control streams were found for mean, maximum, and minimum temperatures ($P>0.05$, univariate analysis of variance (ANOVA)).

2.2. *Thiacloprid application and monitoring*

Thiacloprid (generic name (CA) [3-[(6-chloro-3-pyridinyl) methyl]-2-thiazolidinylidene] cyanamide, CAS number 111988-49-9) belongs to the group of neonicotinoid insecticides. Biological activity of neonicotinoids is based on their interference with the nicotinic acetylcholine receptors and, therefore, they exhibit specific activity against the insect nervous system (Tomizawa and Casida, 2005). Among neonicotinoids, thiacloprid is a new and promising insecticide active against various chewing and sucking pests (Elbert et al., 2001).

Thiacloprid was obtained from Agrar–Handel und Transport (Schafstädt, Germany) as the commercial formulation Calypso (suspension concentrate) with 480 g/L of the active ingredient (Bayer CropScience, Langerfeld, Germany). Nominal concentrations of the three treatment levels were 0.1, 3.2 and 100 μg/L in terms of active ingredient for the low, medium, and high treatments respectively. Throughout the paper we refer to these nominal concentrations.

Thiacloprid has high water solubility (water solubility and log octanol–water partition coefficient (log K_{OW}) of active ingredient at 20 °C is 185 mg/L and 1.26, respectively) (USEPA, 2003). One litre of stock solution was prepared for each

channel at the respective concentrations by diluting the toxicant formulation with distilled water. The stock solutions were poured into the water reservoirs installed below the streams (see above for the mesocosm system description) to dilute the toxicant and make the input gradual. This was done to simulate contamination due to spray drift and surface water runoff, which represent a relevant input path for small streams in agricultural areas (Liess et al., 1999; Neumann et al., 2002). The contamination was performed 18 May 2006.

Exposure to thiacloprid was monitored in the high- and medium-concentration streams using spot water samples taken 48 h, 120 h and 264 h after contamination (measurements at low concentration have not been performed due to technical difficulties). Two 200-ml samples were taken per each channel (in up- and downstream sections, non-vegetated areas) that resulted in 4 samples per each concentration (except the sampling of the channels treated at 100 μg/L performed 264 h after contamination, Table 2). The samples were solid-phase-extracted immediately after sampling using 6 ml Chromabond Easy columns (Macherey-Nagel, Düren, Germany) preconditioned with 6 ml methanol. The columns were eluted with 12 ml acetonitrile-ethylacetate (1:1 v/v) and gently evaporated to 300 μl under nitrogen. Analytical recovery was 82% with 18% standard deviation ($n=3$) for 200 ml of spiked water samples. All solvents used were of HPLC-grade and obtained from Merck KGaA (Darmstadt, Germany).

All analyses were conducted by high-performance liquid chromatography (Agilent 1100 series, Agilent Technologies Germany, Boeblingen, Germany) using a XDB-C18 column (150×2.1 i.d., Agilent Technologies) and an Agilent 1100 liquid chromatograph/mass selective detector (LC/MSD) for quantification. The limit of detection (LOD) for the concentration in the water phase was 0.03 μg/L. The measurements were performed by UFZ—Helmholtz Centre for Environmental Research (Leipzig, Germany).

2.3. *Complementary experiment on thiacloprid dynamics in the stream system*

In order to better investigate the temporal dynamic of thiacloprid exposure in the experimental streams a complementary experiment was conducted in May 2007. Set up of this experiment was identical to the main experiment described above concerning the nominal concentrations, and the methods of preparation of stock solutions, contamination, and water sampling (exception: 2-L samples were taken for concentrations ≤0.1 μg/L). Thiacloprid residues were monitored until the concentrations in the water phase decreased below limit of quantification (Table 3).

Table 2 – Residue analysis of thiacloprid

Time after contamination (h)	Mean measured concentrations ± standard deviation ($n=4$) [a] at different time-points after contamination (μg/L)		
	Nominal concentration (μg/L)		
	0.1	3.2	100
48	NM	3.55 ± 2.80	36.64 ± 10.37
120	NM	1.29 ± 1.61 [b]	6.48 ± 1.43
264	NM	NM	5.43 ± 0.75 [b]

NM—not measured.
[a] two samples in each of the two channels.
[b] $n=2$.

Table 3 – Residue analysis in the complementary experiment on thiacloprid dynamic

Time after contamination (h)	Mean measured concentrations ± standard deviation ($n=4$) [a] at different time-points after contamination (μg/L)		
	Nominal concentration (μg L^{-1})		
	0.1	3.2	100
4	0.08 ± 0.02	2.83 ± 0.16	76.33 ± 10.03 [b]
10	NM	NM	65.67 ± 11.91 [b]
48	0.05 ± 0.01	1.28 ± 0.13	35.25 ± 14.24
120	0.02 ± 0.02	0.24 ± 0.20	12.23 ± 9.61
216	0.02 ± 0.03 [c]	0.05 ± 0.05	2.36 ± 2.62
312	<0.01 [c]	<0.01	0.6 ± 0.61
480	<0.01 [c]	<0.01	0.09 ± 0.14
648	NM	NM	<0.01

NM—not measured.
[a] two samples in each of the two channels.
[b] $n=6$, three samples in each of the two channels.
[c] $n=2$, one sample in each of the two channels.

Measurements were performed with liquid chromatography (high-performance liquid chromatography system with Diodenarray Detector II Series 2000, binary pump, autosampler, column oven (30C), Perkin Elmer, Wellesley, MA, USA). The injection volume was 100 μl, dissolved in 25% acetonitril/water solution with gradient-grade pump program. The detection limit was 0.01 μg/L. The column LiChrospher 60, RP-select B, 5 μm (Merck, Darmstadt, Germany) was used for separation. The LOD for a sample was 0.01 μg/L.

2.4. *Invertebrate community sampling*

2.4.1. *Aquatic sampling*

Aquatic macroinvertebrates were sampled using a metal frame designed to cover a 15×15 cm area of the stream bottom. This frame, shaped like a short square pipe, has 20-cm-high walls and 15×15 cm openings at the bottom and top. During sampling all macrophytes were removed from the sampled area by hand and washed and checked for macroinvertebrates in a white plastic cuvet. Subsequently the water column was sieved and sediments were collected by small hand net (60×55 mm frame, 500 μm mesh) and examined for macroinvertebrates in the white cuvet. During each sampling four samples were taken from each experimental channel at up-, middle-up-, middle-down-, and downstream sections respectively. Each sample was taken from the bottom area including both macrophytes and non-vegetated substrate.

Except for the first sampling, made in September 2005 (34 weeks before contamination), the animals were identified in situ and put back in the stream. During the first sampling all the macroinvertebrates were preserved in 90% ethanol and identified in the laboratory. During subsequent samplings the same procedure was applied when required (e.g. new species

found that could not be identified in situ). Most of the Ephemeroptera, Odonata, Plecoptera, Trichoptera, Heteroptera, Coleoptera, Isopoda and Amphipoda were identified to the species level, while in all other taxonomic groups the identification level varied from species to family. Oligochaeta was identified at the class level only. The samplings were performed at the following time periods with respect to the contamination event: −34, −8, −4, −1, 1, 3, 10, 17, and 27 weeks.

2.4.2. *Emergence traps*

To assess the effect of the toxicant on emergence of merolimnic insects, 6 emergence traps were installed on each stream mesocosm. Each emergence trap was constructed as a pyramid-shaped net that covers approximately 0.165 m^2 of the stream surface area and is 0.76 m in height. The traps consist of a wood frame covered with net (0.7 mm mesh) and a plastic cone installed on the top of the trap. The cone is covered by a plastic 1-L bottle. The whole system was designed to collect all emerged insects in the plastic bottle installed on the top of the trap.

Emerged insects were counted and identified in situ three times per week from 17.05 to 30.09.2006, when insect emergence almost completely ceased. When necessary, insects were preserved in ethanol (representatives of Odonata were fixed with acetone and dried) and identified in the laboratory. Most of the Ephemeroptera, Odonata, Plecoptera, and Trichoptera were identified to the species level, while in Diptera the identification level varied from species to family (appendix table).

2.5. *Data analyses*

The experimental design includes 16 independent streams and four treatment levels (control, 0.1, 3.2, and 100 μg/L) with two replicates for each concentration level and ten for the control (regression experiment design, e.g. Van Wijngaarden et al., 1996). The relatively high number of control replicates was used to allow usage of the Monte Carlo permutation test following multivariate ordination techniques (described below). Data from separate samples taken from the same stream at a particular time-point was pooled to avoid pseudoreplication. Data from emergence traps collected three times per week were pooled for each week and then these weekly values were used to derive a value per month as the arithmetical mean. This was done to provide discrete data points comparable to those obtained in aquatic samples.

To give an overview of the thiacloprid effect on the macroinvertebrate communities the widely employed univariate parameters abundance and taxa richness were used. Abundance was measured as $\ln(x+1)$ transformed number of individuals per square meter. Taxa richness was measured as number of taxa (species or other lowest possible taxonomic category) per sample. Specific "regression" experimental design adopted for multivariate statistical methods (described below) makes difficult comparison of different treatment levels (checking of variance heterogeneity is impossible, tests suitable for such situation are not sensitive and type II error (possibility to find no difference between different populations) is highly probable). Therefore, abundance and taxa richness were plotted against time to only visually inspect for relationship (e.g. Van den Brink et al., 1996) (Fig. 1).

To test for significance of the toxicant's effect on particular species (two species only, explained below) we used ANOVA followed by Games–Howell and Tamhane post-hoc tests. These tests are robust with respect to the potential deviations from normality or variance homogeneity (Zar, 1996). In particular, Tamhane test exhibits good type I error rate (i.e. low probability to find difference between identical populations) and power properties. This conservative test was applied to confirm statistical significances taking into account low number of contaminated replicates.

The community response to the contamination was analysed using the Principal Response Curve (PRC) method and a set of Redundancy Analyses (RDA) performed for the different sampling time-periods. The PRC method is a multivariate technique specially developed for the analysis of data obtained in experimental community response studies. It is based on the RDA ordination technique, the constrained form of principal component analysis (Van den Brink and Ter Braak, 1999). Statistical significance of the PRC models, in terms of displayed treatment variance, was tested by Monte Carlo permutation tests performed for the entire time series in the RDAs from which the PRCs were obtained, using an *F*-type test statistic based on the eigenvalue of the components (Van den Brink and Ter Braak, 1999; Leps and Smilauer, 2003).

Prior to all the multivariate analyses species abundances were $\ln(10x+1)$ transformed, where x stands for the abundance value. This was done to down-weight high abundance values (for rationale see Leps and Smilauer, 2003). The PRC technique was used to analyse the entire process of community development before and after contamination. This statistical technique was applied for (i) the whole community and also (ii) separately for the short-living (multivoltine, life-cycle <1 year) and long-living (uni- and semivoltine, life-cycle ≥1 year) taxa, to understand the recovery dynamics of species having different life-cycle durations. Data from insect emergence traps was analysed separately from the aquatic data, by the PRC method only. In this analysis the entire assemblage of emerged insects was assessed.

The RDAs with toxicant concentration ($\ln(x+1)$ transformed) used as only one explanatory variable were applied in order to test the statistical significance of toxicant effects on the community structure at different toxicant concentrations and different time-points using the Monte Carlo permutation test, and therefore to infer the Lowest- and No-Observed-Effect Concentration (LOEC and NOEC respectively). The latter type of test was performed by testing every concentration level against the control. Community LOEC is defined here as the lowest toxicant concentration at which a significant difference from the control is detected for the community. Similarly, NOEC is defined as the highest concentration at which the effect is insignificant (Newman and Unger, 2003).

The applicability of the Monte Carlo permutation test to assess the significance of separate treatments in experimental community response studies is frequently restricted by a small amount of replicates, as few permutation possibilities cannot yield *P*-values lower than adopted α-level (discussed in Van den Brink and Ter Braak, 1999). In the present study the lowest amount of permutation possibilities available for the model with one concentration level (2 replicates) and control (10 replicates)

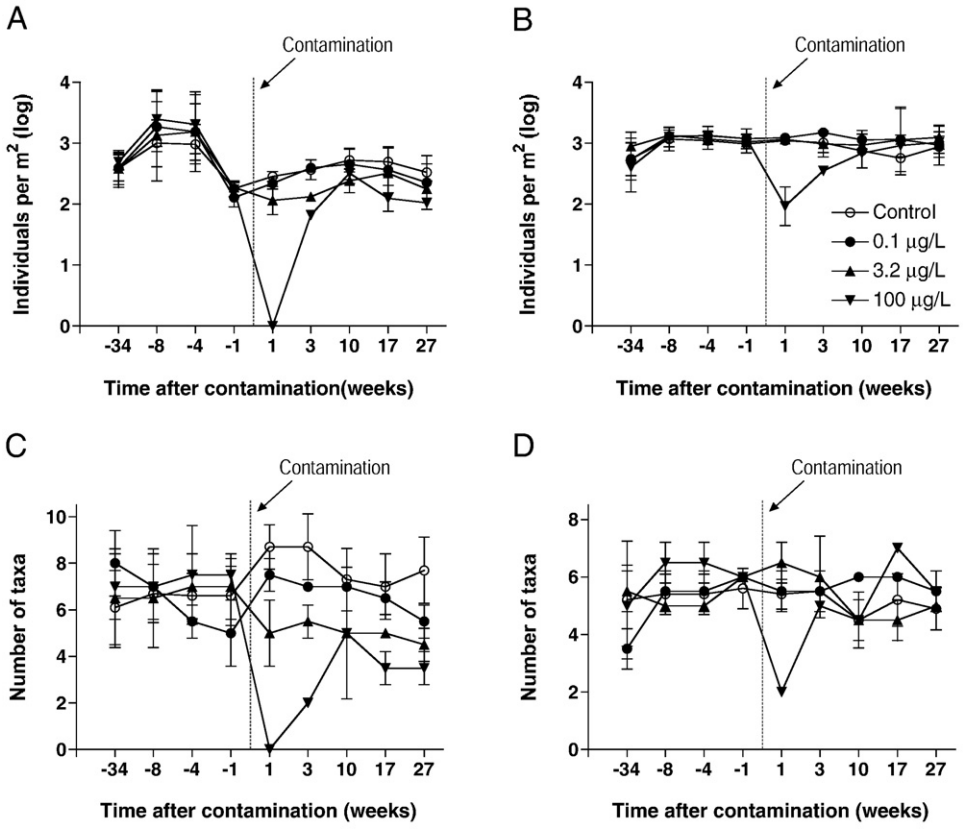

Fig. 1 – Dynamics of abundance and taxa richness of aquatic macroinvertabrates (log(x+1)-transformed number of individuals per square meter and number of taxa respectively). Abundance of insects (A) and non-insects (B), taxa richness of insects (C) and non-insects (D).

was 66 (12!/[2!10!) and a corresponding lowest possible permutation-based P-value was 0.015 (1/66). Hence it was possible to use here the set of Monte Carlo permutation tests to test significance of effects of different concentrations and to infer community LOECs for particular time points.

All multivariate statistical analyses were made using the program CANOCO 4.5 for Windows (Wageningen, the Netherlands) according to the available guides (Ter Braak and Smilauer, 2002; Leps and Smilauer, 2003).

Recovery was considered to be achieved when statistical tests for the first time failed to detect a significant difference between contaminated and control mesocosms under condition that a significant effect was not detected later during the observation period.

3. Results

3.1. *Thiacloprid exposure dynamic*

In the main experiment thiacloprid concentrations were monitored during the eleven days after exposure (Table 2). As explained above, in order to better examine dynamic of the toxicant in water a complementary experiment was conducted. In this latter experiment thiacloprid was monitored until complete disappearance from the water phase (27 days, Table 3). In both experiments the measured concentrations of thiacloprid were within the range of nominal concentrations after contamination (Tables 2 and 3). The toxicant concentration in the streams was characterised by an initial rapid drop of the concentration followed by a lower decline in concentrations. Results of the measurements suggest that (i) there was no accumulation (at concentrations≤LOD) of thiacloprid in the water phase after 27 days following contamination (Table 3), and (ii) the exposure profile was of the pulse type (Tables 2 and 3) that is similar to pulse exposures observed in streams in the field with peak pesticide concentrations lasting for hours (Richards and Baker, 1993; Liess et al., 1999; Leu et al., 2004). Information about behaviour of thiacloprid in surface water is limited (Krohn, 2001), and detailed comparison of the observed exposure with other studies is problematic.

3.2. *Abundance and taxa richness*

A total of 35 macroinvertebrate taxa were identified for the mesocosm systems (appendix table). Only 21 out of these 35 taxa were found in more than two streams and on more than one occasion. Only these taxa were considered in the multivariate statistical analyses reported below. In terms of the numbers of taxa the richest taxonomic group was insects (26 taxa). The dominant species in all the streams were the isopod *Asellus aquaticus* and blackfly larvae *Simulium latigonium* (relative abundances were up to 70 and 75% respectively). The proportion of long-living taxa having no more than one generation per year was 54% of the overall taxa richness and

47% of the established taxa (21 species mentioned above, appendix table).

Abundance and taxa richness of insect and non-insect taxa were considered separately, as thiacloprid is much more toxic to insects than to other invertebrates, including crustaceans (Beketov and Liess, 2008a,b). The effect of thiacloprid on insect abundance was stronger than on non-insect macroinvertebrates (Fig. 1A and B). Total insect abundance recovered after 10 weeks following the contamination (Fig. 1A). In contrast to the abundance, no recovery was observed for insect taxa richness during the entire observational period at 3.2 and 100 μg/L. (Fig. 1C). Non-insect abundance and taxa richness only showed a transient reduction following contamination (Fig. 1B and D).

Abundance and taxa richness of emerged insects was suppressed at 100 and 3.2 μg/L and 100 μg/L respectively (Fig. 2A and B). Full recovery of these two parameters was observed after 4 and 8 weeks following the contamination respectively.

3.3. *Community structure and LOEC*

The diagram of the first PRC of the aquatic macroinvertebrates (Fig. 3) shows small variation in the pre-treatment period and clear concentration-dependent deviations from the control after the thiacloprid application. Statistical significance of the first PRC was confirmed by the permutation test ($P=0.01$). The second PRC was not statistically significant ($P>0.05$), and therefore is not considered here. Taxa indicated with a higher species scores (b_k), shown on the right side of the PRC diagram (e.g. *S. latigonium*, *Cloeon dipterum*, Fig. 3), decreased in abundance more severely at the higher toxicant levels. In contrast, taxa with negative scores (Oligochaeta and *Planorbis* sp.) increased at the higher toxicant levels. As in the PRC diagram constructed for the aquatic macroinvertebrates (Fig. 3), the first PRC of the emerged insects data set shows relatively small variation in the pre-treatment period and clear, but short-term (until 8 weeks after contamination) concentration-dependent deviations from the control after the contamination (not shown). The first PRC for emergence data set was statistically significant ($P=0.01$); the second was not ($P>0.05$).

Results of the Monte Carlo permutation tests subsequent to the RDAs performed for aquatic macroinvertebrates and emerged insects are summarised in Tables 4 and 5 respectively. Significances derived in the permutations for aquatic macroinvertebrates are also reported in the PRC diagram (Fig. 3).

Results of these permutation tests show that the effect of the toxicant on aquatic communities was significant at concentrations 3.2 and 100 μg/L after 1 and 3 weeks following contamination. During the following time (10–17 weeks after contamination) significance of the effect was only found for the whole model (i.e. permutation all concentrations and control series), which means that significant effect of toxicant cannot be attributed to any separate concentration (Table 4, Fig. 3). After 27 weeks following contamination, the effect of the toxicant again became significant at 3.2 and 100 μg/L (Table 4, Fig. 3).

These results suggest that aquatic macroinvertebrate community structure did not recover until the end of the observation period, as at concentration 3.2 μg/L a significant effect of the toxicant was detected 27 weeks after the contamination. The community LOEC for the latest observation period (27 weeks) is equal to 3.2 μg/L (Table 4).

For the assemblage of emerged insects a significant effect of the toxicant at the concentrations 3.2 and 100 μg/L was found after 1 week following contamination only. At four weeks after the contamination, the effect was significant at 100 μg/L only; and no significant differences were found during the entire subsequent observation period at any concentrations (Table 5).

3.4. *Effect dynamics of short- versus long-living taxa*

The species comprising the macroinvertebrate communities in the present experiment are characterised by contrasting life-cycle patterns such as seasonal dynamics and life-cycle duration. For example, two extremely contrasting dynamics of taxa having different life cycles, namely the abundance dynamics of short-living Chironomidae and the long-living stonefly *Nemoura cinerea*, are shown in Fig. 4. Representatives of the Chironomidae are known to be short-living and

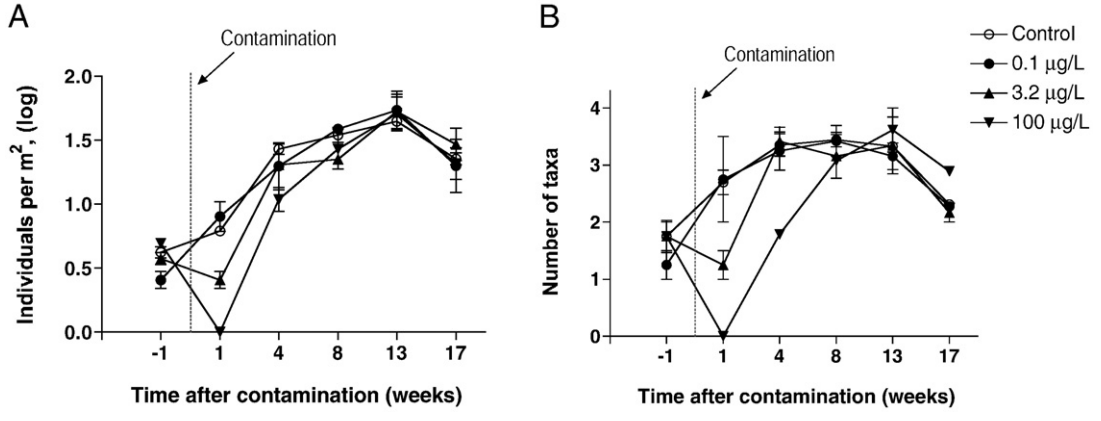

Fig. 2 – Dynamics of abundance (A) and taxa richness (B) of emerged insects (log(x+1)-transformed number of individuals per square meter and number of taxa respectively).

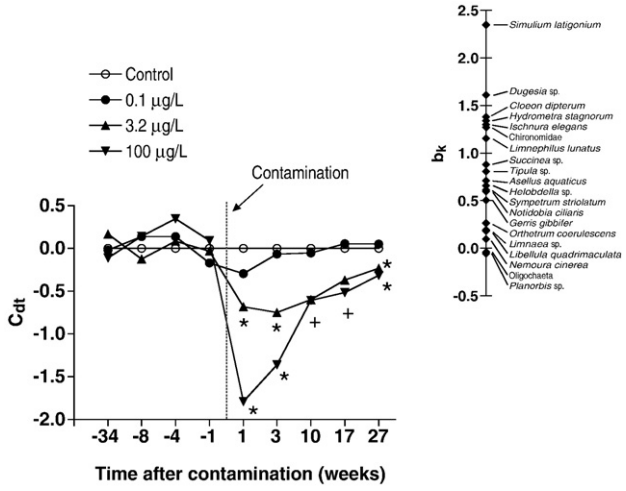

Fig. 3 – Principal Response Curves (PRC) indicating the effect of insecticide thiacloprid on macroinvertebrate community. The vertical axis represents the difference in community structure between treatments and the control expressed as regression coefficient (C_{dt}) of the PRC model. The species score (b_k) can be interpreted as a correlation of each species with the response given in the diagram (taxa indicated with a higher scores show a greater decrease in abundance at the higher toxicant levels). Asterisks indicate significant ($P<0.05$) effect of factor toxicant at particular concentrations tested by Monte Carlo permutation test followed RDA. Plus marks denote significance of the same factor in the whole model in case no particular concentrations yielded statistical significance.

multivoltine (Liess et al., 2008). It is clear from Fig. 4A that although abundance of this taxon was initially severely affected by the contamination, it fully recovered after 10 weeks following contamination. In contrast to Chironomidae, the stonefly *N. cinerea* has only one generation per year (Liess et al., 2008). In addition, the egg and larval development of this species is relatively slow, which makes it difficult to detect the larvae of this stonefly during some time (usually 1 to 3 months) after the flight period in April–May (Fialkowski, 1986; Brittain and Lillehammer, 1987). In the present investigation no larvae of *N. cinerea* were found during the period from 1 week before to 17 weeks after contamination (Fig. 4B). However, when the *N. cinerea* larvae became detectable (27 weeks after contamination) an apparent effect of the toxicant on this species was found (Fig. 4B). The same dynamics were recorded for the semivoltine mayfly *Ephemera vulgata*; however, this species was less abundant, and during the time period before contamination it was found as imago in two streams only (control and 0.1 µg/L, not shown).

This example suggests that (i) during the post-contamination period different species contribute differently to the observed overall community effect (Fig. 3, Tables 4 and 5), and (ii) recovery dynamics of species depend on their life-cycle traits.

To reveal differences in effect-and-recovery dynamics between short- and long-living organisms, the PRC analyses were done separately for assemblages of multivoltine and uni- as well as semivoltine macroinvertebrate taxa (Fig. 5A and B). These two diagrams show distinctly different post-contamination assemblages' responses. The short-living assemblage exhibits a strong initial effect and complete recovery after 10 weeks following contamination (Fig. 5A). In contrast, the long-living taxa's PRC demonstrates long-term effect and no recovery during the entire period of observation (27 weeks, Fig. 5B). The first PRCs for both of the assemblages were statistically significant ($P=0.002$), whereas the second PRCs were not ($P>0.05$).

As in the analyses of the entire community described above, the PRCs for short- and long-living assemblages were supplemented with a set of RDAs with Monte Carlo permutation tests in order to test statistical significance of factor toxicant at separate time-periods and concentrations. Significances derived in these permutation procedures are reported in the PRC diagrams (Fig. 5A and B) as described above for the analyses of the entire aquatic macroinvertebrate community.

Table 4 – Results of the Monte Carlo permutation tests followed the Redundancy Analyses for different sampling dates (data from aquatic samples)

Time after contamination (weeks)	P-values				LOEC (µg/L)	NOEC (µg/L)
	Complete model	Separate concentrations				
	All concentrations	0.1 µg/L	3.2 µg/L	100 µg/L		
−34	NS	NA	NA	NA	NA	NA
−8	NS	NA	NA	NA	NA	NA
−4	NS	NA	NA	NA	NA	NA
−1	NS	NA	NA	NA	NA	NA
1	0.002	NS	0.02	0.024	3.2	0.1
3	0.002	NS	0.032	0.024	3.2	0.1
10	0.009	NS	NS	NS	>0.1*	>0.1*
17	0.028	NS	NS	NS	>0.1*	>0.1*
27	0.008	NS	0.036	0.048	3.2	0.1

NS—not significant.
NA—not applicable.
*LOEC and NOEC cannot be precisely determined, as significant effect was only found for all concentrations together (complete model), but no significant effect was found for any of the separate concentrations.

Table 5 – Results of the Monte Carlo permutation tests followed the Redundancy Analyses for different sampling dates (data from insect emergence traps)

Time after contamination (weeks)	P-values				LOEC (μg/L)	NOEC (μg/L)
	Complete model	Separate concentrations				
	All concentrations	0.1 μg/L	3.2 μg/L	100 μg/L		
−1	NS	NA	NA	NA	NA	NA
1	0.002	NS	0.02	0.024	3.2	0.1
4	0.006	NS	NS	0.024	100	3.2
8	NS	NA	NA	NA	>100	≥100
13	NS	NA	NA	NA	>100	≥100
17	NS	NA	NA	NA	>100	≥100

NS—not significant.
NA—not applicable.

Remarkably, the deviation from control observed for the long-living species assemblage was associated not only with negatively affected sensitive insect species, but also with a strong positive and presumably indirect effect on the gastropod *Limnaea* sp. (Fig. 5B).

3.5. *Effect on the stonefly* N. cinerea *at concentration* 0.1 μg/L

As mentioned above, among the taxa affected by the toxicant there was one species, namely the stonefly *N. cinerea*, absent in all contaminated mesocosms, including the series with

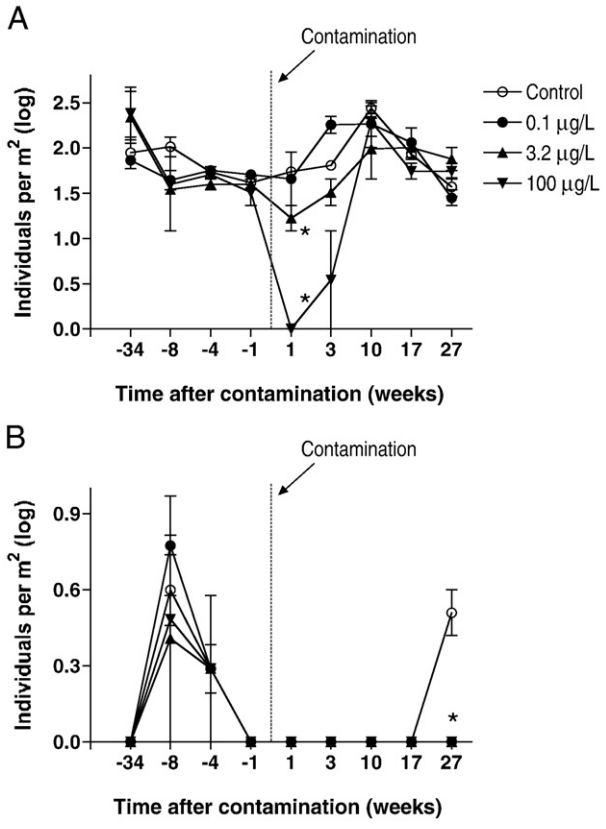

Fig. 4 – Abundance dynamic of two taxa having different life cycles and showing different effect-and-recovery dynamics: short-living multivoltine Chironomidae—strong immediate effect and fast recovery (A), univoltine stonefly *Nemoura cinerea* (B)—strong delayed effect and slow recovery. The latter species was hardly detectable during summer due to slow egg and larval development. Asterisks indicate significant ($P<0.05$, ANOVA, confirmed by both Games–Howell and Tamhane post-hoc tests) differences from the controls.

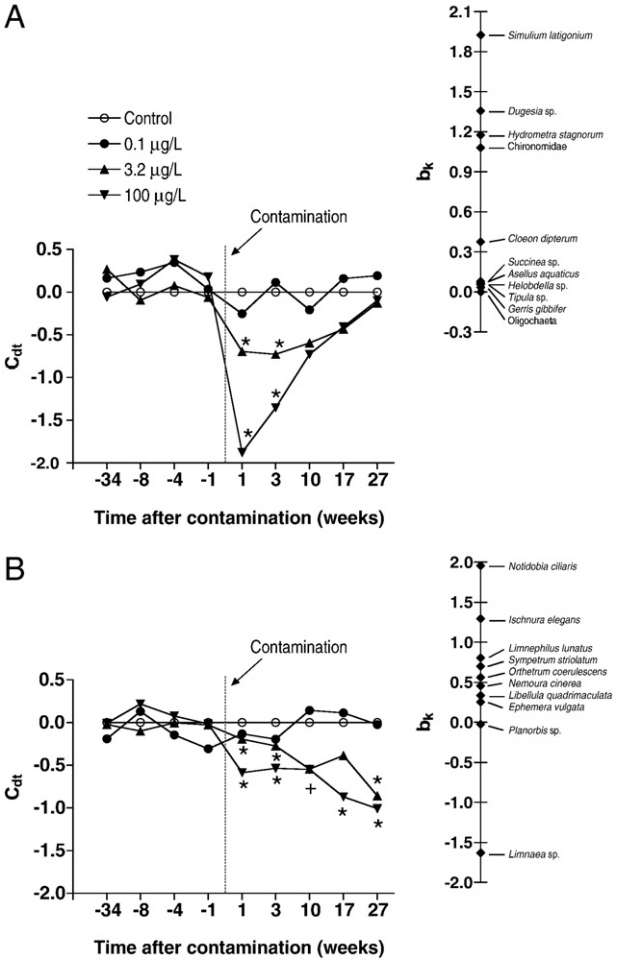

Fig. 5 – Principal Response Curves (PRC) indicating the effect of insecticide thiacloprid on short-living (A) and long-living (B) assemblages of macroinvertebrates (multivoltine and uni- and semivoltine taxa respectively). Explanations in Fig. 3.

lowest tested concentration 0.1 μg/L (Fig. 4B). As explained above, this species after the contamination was found in the late autumn sampling only (27 weeks after contamination). No individuals of this species were found during summer in any treatment including control, obviously because larvae and eggs were too small to be detected (Fialkowski, 1986; Brittain and Lillehammer, 1987). However, prior to the springtime contamination larvae of *N. cinerea* were detected in 9 of the 16 experimental streams, including the streams, which later were contaminated at concentration 0.1 μg/L. Hence this species existed in the streams, which were contaminated with 0.1 μg/L before the experimental contamination and disappeared from these streams after the contamination. In contrast, *N. cinerea* was well established in the control after the contamination period as detected during the autumn sampling. At this sampling period the larvae of *N. cinerea* were detected in 8 out of all 10 control streams with maximum abundances 22 individuals per square meter. Evidently the aquatic stages of this species were present in the water during the contamination period, because, having emerged about 5 weeks before contamination, the adults have certainly completed oviposition well before the contamination occurred. Effect of the toxicant on abundance of *N. cinerea* at concentration 0.1 μg/L was statistically significant ($P<0.05$, ANOVA, both Games–Howell and Tamhane post-hoc tests, Fig. 4B). All this suggests that the absence of *N. cinerea* in streams contaminated at 0.1 μg/L is caused by the toxicant, although the effect was observed only in the particular season and after the considerable time period following contamination. Importantly, this effect was confirmed with conservative Tamhane test that is robust with respect to the potential deviations from normality and variance homogeneity, and exhibits good type I error rates (i.e. low probability to find difference between identical populations) and power properties.

N. cinerea comprise approximately 5% of the total number of established macroinvertebrate species, i.e. taxa for which the toxicant effect could be assessed (21 taxa, the taxa found in more than two streams and for more than one time period, appendix table), and comprise on average 1% of the total abundance (maximum abundance is up to 3%, late autumn sampling, 27 weeks after contamination in the control).

Besides *N. cinerea*, there was one more species, namely the mayfly *E. vulgata*, present in the control streams only (27 weeks after contamination). However, this species was present in 4 out of all 10 control streams, and therefore probability of random non-occurrence of this species in the streams was too high (for one stream $P=0.6$, for two streams $P=0.36$) to test the effect significance with ANOVA and the post-hoc tests.

4. Discussion

4.1. *Comparison of concentrations causing effects in the mesocosm with organism-level toxicity data*

As mentioned, for pesticides there is some uncertainty regarding the levels of concentration that cause effects on aquatic non-target organisms in higher-tier test systems. Hence it is interesting to compare the concentrations causing effects in the present mesocosms with available laboratory-generated organism-level toxicity data.

Thiacloprid is known to be selectively toxic to insects (Beketov and Liess, 2008a,b). The lowest acute (observation time 96 h) LC50 known for pulse (24 h) exposure to thiacloprid is 7.04 (6.45–7.7) μg/L (95% confidence interval in parentheses, found for Trichoptera larvae *Notidobia ciliaris*, Beketov and Liess, 2008a). This LC50 is not significantly different from LC50s found in similar conditions for such sensitive aquatic insects as mosquito and blackfly larvae (7.1 and 7.79 μg/L for *Culex pipiens* and *S. latigonium* respectively, Beketov and Liess, 2008a). Hence the concentration level 7 μg/L is not an outlying value that is substantially lower than LC50s found for other sensitive species. Importantly, these LC50s were found for the short-term exposure (24 h) that is more comparable to the exposure observed in the mesocosms in the present study (rapid concentration decline, Tables 2 and 3) than to the continuous exposure profiles usually used in standard tests. In laboratory tests with continuous exposure (e.g. 96 h) a significantly slower concentration decline is expected than in the streams, as thiacloprid is resistant to degradation by photochemical reactions and hydrolysis, and is mainly eliminated by microbial metabolism (Krohn, 2001).

The lowest LOEC for entire community obtained by Monte Carlo permutation test in the present study is 3.2 μg/L (Tables 4 and 5). This value is approximately 2 times lower than the bottom 95% confidence interval of the lowest known acute LC50. This level is within the range of effective concentrations reported in the mesocosm studies with non-persistent insecticides as reviewed by Van Wijngaarden et al. (2005).

Although the community LOEC, found by Monte Carlo permutation test, was defined as 3.2 μg/L, among the taxa present in the mesocosms there was the stonefly *N. cinerea* that was absent in all contaminated streams including the series with the lowest tested concentration 0.1 μg/L, but were found in the control streams only (Fig. 4B). Hence, for this species only the long-term (27 weeks) LOEC in mesocosms is below 0.1 μg/L. This concentration level is 70 times lower than the lowest known laboratory-generated LC50 of thiacloprid (Beketov and Liess, 2008a). This level is lower than those reported in the mesocosm studies with non-persistent insecticides (Van Wijngaarden et al., 2005), but higher than those found by some microcosm and mesocosm studies focused on the chronic post-exposure effects in sensitive species (Lozano et al., 1992; Liess and Schulz, 1996; Liess, 2002; Beketov and Liess, 2005) and the field studies (Liess and von der Ohe, 2005; Schäfer et al., 2007).

The mechanisms associated with toxicant effects at concentrations 70 times below the acute LC50 are currently unclear. One possible explanation may be that *N. cinerea* is more sensitive to thiacloprid than the sensitive insects tested in the laboratory conditions (larvae of caddisflies, mosquitoes, and blackflies, Beketov and Liess, 2008a), because stoneflies (Plecoptera) are known to be exceptionally sensitive to organic toxicants (Wogram and Liess, 2001). This hypothesis can be tested by future laboratory toxicity tests with larvae of *N. cinerea* or similar stonefly species, as currently no toxicity data exists for Plecoptera and thiacloprid. Other explanations may be that effect on *N. cinerea* at the concentration 0.1 μg/L might result from an interplay of many factors, such as the young age of the exposed individuals (Stark and Banken, 1999; Breitholtz et al., 2003; Pettigrove and Hoffmann, 2005 and references

therein), downstream drift initiated by the toxicant (for thiacloprid see Beketov and Liess, in press), additional stress due to food limitation (Beketov, 2004; Pieters et al., 2005), predation stress (Relyea, 2003; Beketov and Liess, 2006), and effects of abiotic factors (e.g. UV can significantly increase sensitivity to toxicants in field conditions as compared to the laboratory tests, Duquesne and Liess, 2003). Obviously, the relatively long post-exposure observation period is necessary to detect effects at such low concentrations, as effects at concentration levels more than 100 times lower than the acute EC50s were shown in the chronic (almost entire life cycle) microcosm experiments only (Liess and Schulz, 1996; Liess, 2002; Beketov and Liess, 2005). Further investigations are required for understanding the underlying mechanisms of toxic effects at such low concentrations.

For environmental risk assessment of toxicants the two hypotheses given above concerning the effect at the very low concentrations imply that (i) the range of species tested in laboratory toxicity tests should be representative to sufficiently consider the among-taxa variability in sensitivity, and (ii) the realistic environmental context including biotic and abiotic factors, which can exacerbate toxic effects, should be taken into account.

4.2. *Recovery dynamics: importance of species' life-cycle duration and seasonal dynamics*

As mentioned above, for pesticides there is some uncertainty not only regarding effect concentrations, but also concerning recovery duration of aquatic communities impaired by insecticides. As reviewed by Van Wijngaarden et al. (2005), most of the previously published mesocosm studies with non-persistent insecticides have shown that recovery is already completed within two months after contamination. However, a few long-term mesocosm experiments have revealed that even a single short-term exposure to pesticides may result in long-term and permanent elimination of long-living species (Van den Brink et al., 1996; Caquet et al., 2007).

The present study shows that a single pulse contamination at the concentration level close to the acute laboratory-generated LC50s can result in long-term alteration of community structure when long-living species are present in a high proportion (i.e. 50% of overall taxa richness) comparable with natural streams (e.g. 40–80%, own calculations with data from Schäfer et al., 2007). Importantly, the present results show that within the levels of effect concentrations, time for recovery of the affected organisms depends on the life-cycle duration, but not on the toxicant concentration. Thus, short-living (mulivoltine) species recovered already after 10 weeks following contamination, irrespective of the concentrations (Fig. 5A). In contrast, long-living taxa did not recover until the end of the observation period at the effective concentrations (for community structure 3.2 and 100 μg/L, Fig. 5B).

Obviously, long-living insects present in this mesocosm system may recover only after the flying period in the following year. This may be when imagoes emerged in uncontaminated streams will oviposit in the impaired streams. However, in river systems recolonisation of affected stream parts occurs not only through aerial dispersal, but also through the drift of aquatic stages of merolimnic insects and fully aquatic animals from unaffected upstream reaches. Several studies have shown that the presence of undisturbed upstream reaches significantly reduces pesticide effects on invertebrates and facilitates recovery of contaminated streams (Hatakeyama and Yokoyama, 1997; Liess and von der Ohe, 2005; Schäfer et al., 2007; Schriever and Liess, 2007; Schriever et al., 2007). All this suggests that prediction of ecosystem recovery after pesticide contamination should consider life-cycle traits of sensitive species and spatial isolation of the affected area from undisturbed ecosystems.

The endpoint insect emergence exhibited relatively rapid recovery in terms of taxonomic structure as compared to the aquatic communities. No significant effect of the toxicant on the emerged insects was found after 8 weeks following contamination (Table 4). This rapid recovery is obviously caused by strong prevalence of multivoltine taxa in the assemblage of emerged insects (Chironomidae and Simuliidae). These taxa have several emergence periods during the year and these periods are extended and overlapping.

The processes underlying the long-term community structure alteration observed in the present study included not only elimination of sensitive long-living species, but presumably also a positive indirect effect on the gastropod *Limnaea* sp. (Fig. 5). Such positive effects of pesticides on gastropods were observed previously in mesocosm (reviewed by Fleeger et al., 2003) and field studies (Liess and von der Ohe 2005). Mechanism of this effect may be explained by the reduced competition that results from elimination of more sensitive competitors (insects), as was also proposed in previous community-level studies (Fleeger et al., 2003).

5. Conclusion

We conclude that in mesocosms the long-term (7 month) LOEC calculated for the entire community by multivariate statistical methods can be found at concentrations in the range of the acute LC50 of sensitive species. However, it cannot be excluded that effect on a minority of species can occur at concentrations far below the laboratory-generated acute LC50.

Concerning the post-exposure recovery, we conclude that within the levels of effect concentrations, recovery of the affected organisms may be predominantly dependent on the life-cycle duration, and not on the toxicant concentration.

In environmental risk assessment realistic prediction of pesticide effects at the community level requires consideration of long-term effects. Prediction of recovery dynamics in communities impaired by pesticides should consider life-cycle duration of the species comprising the communities.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scitotenv.2008.07.001.

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