Controlling *Culex pipiens*: antagonists are more efficient than a neonicotinoid insecticide

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ABSTRACT: Species vulnerability to pesticides depends on physiological sensitivity, the potential to recover, and the ecological context. We assessed the vulnerability of the mosquito *Culex pipiens* to a repeated treatment with thiacloprid in outdoor microcosms with and without antagonists (competitive and predatory invertebrates). Microcosms were treated repeatedly (three times) with thiacloprid at a concentration of 0.1, 1, or 10 µg/liter. In microcosms without antagonists, the abundance of *Cx. pipiens* larvae decreased moderately after the second and the third exposures to 10 µg/liter thiacloprid. In microcosms with antagonists, the abundance of *Cx. pipiens* larvae declined to approximately zero in the control group and the low concentration treatments during the five weeks of observation. By contrast, the abundance of *Cx. pipiens* larvae temporarily increased at 10 µg/liter thiacloprid after the second and third contamination. We explained this positive effect on the development of *Cx. pipiens* because of the decrease in competition due to the elimination of sensitive antagonists combined with the high recovery potential of *Cx. pipiens*. Based on these results, natural antagonists must be supported for the sustainable control of mosquitoes. ***Journal of Vector Ecology* 43 (1): 26-35. 2018.**

Keyword Index: Thiacloprid, *Culex pipiens*, invertebrate taxa, competitors, predators, mosquito control.

INTRODUCTION

Pesticides in ecosystems can reduce interspecific competition and therefore indirectly increase the population growth of mosquitoes (Foit et al. 2012). A pesticide-induced reduction of predation can also result in a strong resurgence of mosquitoes, as reported by Grigarick et al. (1990) for *Culex tarsalis* at a rice research experimental station. Similarly, following the eradication of predators by the pesticide lambda-cyhalothrin, Dennett et al. (2003) and Lawler et al. (2007) also describe a proliferation of mosquitoes.

The aim of integrated pest management (IPM) is to apply the knowledge of species interactions to selectively kill the target organism and minimize risks to the environment, including those to beneficial and non-target species (Flint et al. 1998). An important option of IPM is the combination of pesticides with natural enemies or antagonists of the pest (Liess and Duquesne 2014). The efficacy of this approach relies on the pesticide causing little harm to pest antagonists.

Thiacloprid is a neonicotinoid insecticide with potential for application in mosquito control (Corbel et al. 2004, Maurya et al. 2012, Ahmed and Saba 2014). Neonicotinoids are known for their high toxicity to insects (Beketov and Liess 2008a) and their low toxicity against several crustaceans that are efficient antagonists of mosquitoes (Beketov and Liess 2008b). These insecticides permanently bind to nicotinic receptors of acetylcholine causing paralysis followed by death (Tomizawa and Casida 2005). In the current study, we explored the effects of thiacloprid on larvae of *Cx. pipiens* at three levels of biological organization. The results of this study will contribute to the body of information about general mechanisms that affect the efficiency of pesticides in mosquito control.

MATERIALS AND METHODS

Two experiments were conducted: a laboratory experiment to study the effects of thiacloprid on larvae of *Cx. pipiens* at the individual level and an outdoor microcosm experiment to investigate the effects of thiacloprid at population and community levels.

Laboratory experiment

Laboratory toxicity tests were conducted to assess the effects of thiacloprid at the individual level on larvae of *Cx. pipiens* and on mosquito larvae antagonists. Antagonists included Cladocera, Ostracoda, Copepoda, and larvae of Chironomidae as important competitors (Kröger et al. 2013, Meyabeme Elono et al. 2010) and larvae of Odonata as important predators (Kumar and Hwang 2006). We adapted the toxicity tests to the concept of Rapid Tests (Kefford 2013). Test organisms were collected from uncontaminated control microcosms that were a part of the "community added" set-up of the outside experiment. Cladocerans included the genera *Ceriodaphnia, Chydorus, Daphnia*, *Simocephalus* and *Scapholeberis*. Copepods were composed of Cyclopoida, and Anisoptera and Zygoptera contributed to Odonata. The Elendt M4 medium was used to prepare the exposure concentrations: 0.1, 1, 10, 100, 1000, 10,000, and 100,000 µg/liter.

The experimental design of this test is summarized in Table 1. We used the suspension concentrate Calypso® 480 g/liter thiacloprid (Agrar-Handel und Transport, Schafstädt, Germany). Test organisms were accommodated in M4 medium under laboratory conditions with a temperature of 20 ± 1° C and a constant photoperiod of 16:8 h (L:D) for 24 h before the experiment. Individuals were continuously exposed to thiacloprid without exchange of the test medium, as in the microcosm experiment. Organisms in the laboratory experiment were fed twice a week. Cladocera, Ostracoda, Copepoda, and larvae of Chironomidae

were fed batch-culture green algae (*Desmodesmus subspicatus*) with a concentration between $12x10^5$ and $123x10^5$ cells/ml (total carbon content between 0.5 and 5 mg/liter). Larvae of Odonata were fed *Cx. pipiens* larvae (five *Culex* larvae/Odonata). The exposure was performed in two types of vessels depending on the size of the test organism. For Copepoda, larvae of Chironomidae, *Cx. pipiens*, and Odonata with a maximal length between 2 and 40 mm, we used 50 ml beakers, each containing 20 ml of test solution. For Cladocera and Ostracoda with a maximal length between 0.4 and 1.5 mm, we used round-bottomed test tubes (soda-lime glass, length 13 cm, diameter 1 cm), each containing 10 ml of test solution. Test organisms were considered affected when they remained immobile to mechanical excitation (e.g., shaking). The total duration of observation ranged from six to 25 days, depending on the life span of taxa during the test. All results are shown in Table 2. For statistical analyses, the six-day EC_{50} was used. The corresponding median effective concentrations (EC_{50}) were calculated using the Trimmed Spearman-Karber method (Hamilton et al. 1977) in the program SPEARMAN, version 1.5 (Montana State University, Bozeman, MT, U.S.A.).

Microcosm experiment

The microcosm investigation occurred outside over 53 days in summer in the experimental area of the Helmholtz Centre for Environmental Research-UFZ, Leipzig (Central Germany). We constituted 32 freshwater microcosms in graduated 90 liter plastic buckets with an opening of 0.5 m; the buckets were buried with two-thirds in the ground to simulate the natural environment of a pond. Each microcosm contained 2 cm of mixed sediments from the natural ponds of Rosslau, Leipzig, and Spreewald (Central Germany). Sediments were oven-dried at 120° C (Heraeus Instruments, Hanau, Germany) for 12 h to minimize contamination with unwanted organisms from sediments. The ponds were filled with 60 liters of tap water.

We divided the 32 microcosms into two set-ups, microcosms with "no community added" and microcosm with "community added." The "community added" microcosms were enriched with invertebrates from the natural ponds of Rosslau, Leipzig, and Spreewald. To concentrate the field-collected invertebrates, approximately 300 liters of pond water was filtered to 1 liter through a 55 µm mesh. This procedure was repeated several times, and the 1 liter subsamples were pooled into a single sample. Each microcosm of the "community added" set-up received 500 ml of this community concentrate. The communities in the microcosms were composed of taxa of Ostracoda, Cladocera, Copepoda, and larvae of insects (i.e., Chironomidae and Odonata). All microcosms were covered with nets during the first five days of the experiment for the added biocoenosis to acclimate and to prevent colonization with mosquitoes. After five days, we opened the microcosms for colonization by local populations of *Cx. pipiens*. Microcosms then remained open during the entire period of the experiment to allow continuous recolonization by adult mosquitoes from the surroundings. One day before the first treatment with thiacloprid, the mean abundance of added invertebrates was 272 (± 54 SE) and 0 individuals/liter in "community added" and "no community added" microcosms, respectively. During the experiment, "no community added" microcosms also contained some larvae of Chironomidae. However, the proportion of Chironomidae in these microcosms was low and averaged 3% of the abundance of *Cx. pipiens*. In "community added" microcosms, the proportion was higher with an average of 8.9% and 39% for Chironomidae and all antagonists, respectively. Hence, the abundance of Chironomidae in the "no community added" microcosms was negligible and not considered in the following data analyses. In all microcosms, we established the food conditions by providing batch-cultured green algae (*Desmodesmus subspicatus*) at a concentration of 3.1 x 10^5 cells/ml (total carbon content of 0.13 mg/liter); algae were obtained from the University of Göttingen, Germany.

Thiacloprid exposure

The starting point of the microcosm experiment was assigned to the first sampling of *Cx. pipiens* and corresponded to "day 0" of our experiment. Microcosms were exposed to thiacloprid on days 5, 19, and 34. The "no community added" and "community added" microcosms were exposed to thiacloprid at the nominal concentrations of 0 (control), 0.1, 1, and 10 µg/liter with four replicates for each concentration and community set-up. A stock solution of thiacloprid at 10 mg/liter was prepared in glass bottles (Calypso® 480 g/liter in distilled water, total volume 2 liters). Volumes of 0.6, 6, and 60 ml of this stock solution were spread on the surface of microcosms to attain the nominal concentrations of 0.1, 1, and 10 µg/liter. The water was then stirred gently with five turns of a 1 cm diameter glass stick. The selected nominal concentrations for the microcosm experiment were based on the lethal median concentrations, LC_{50}s, of two acute toxicity tests with *Cx. pipiens*. Beketov and Liess (2008a) identified a 14-day LC_{50} of 6 µg/liter thiacloprid. We repeated the test at higher concentrations and observed a 5-day LC_{50} of 9.9 µg/liter (unpublished data).

Sampling and monitoring of taxa

The oviposition and larval abundance of *Cx. pipiens* in the microcosms were sampled at least twice per week, with one sampling before and one after each application of thiacloprid. Egg rafts were counted on the water surface. Mosquito larvae were sampled by dipping four times a volume of 250 ml at the edge of each microcosm (adapted standard dipping technique, WHO 1975). The four samples were averaged, and the abundance was expressed as individuals/dip. Counted larvae were immediately returned to the microcosm.

The invertebrate taxa associated with larvae of *Cx. pipiens* were sampled once before and once after each application of thiacloprid. To obtain a representative sampling of the communities, we collected six samples of 250 ml from different sides and depths of each microcosm. The subsamples were pooled to a single sample and gently stirred. One liter of the pooled sample was filtered through a 55 µm mesh and preserved in a 15 ml-flask with a mixture of ethanol and distilled water (ratio 70:30). The remaining part of the pooled sample was returned to the respective pond. In the laboratory, taxa were identified and counted using microscopes, a binocular Nikon SMZ 645 (Tokyo, Japan) and a ZEISS Axiostar (Oberkochen, Germany). We used the following identification keys: Ward and Whipple (1966), Durand and Lévêque (1980), Schwab (1995), Becker et al. (2003), Tachet et al. (2003) and Streble and Krauter (2006).

Table 1. Experimental design of the laboratory toxicity tests with *Cx pipiens* and selected antagonists. Toxicity tests were conducted as Rapid tests with continuous exposure (Kefford 2013).

Taxa	Duration (day)	Replicate	Individuals per replicate	Individual per concentration
Cladocera	9	30	1	30
Copepoda	9	10	1	10
Ostracoda	25	5	5	25
Larvae *Cx. pipiens*	6	10	5	50
Larvae Chironomidae	6	10	1	10
Larvae Odonata	9	10	1	10

Table 2. Mean and range of values for dissolved oxygen (DO), pH, electrical conductivity (EC), total dissolved solids (TDS), and chlorophyll (Chl) for the different concentrations in the "no community" (n =16) and "community added" (n =16) microcosms.

Factor		Control		0.1 µg/liter		1 µg/liter		10 µg/liter	
		mean	min - max	mean	min - max	mean	min - max	mean	min - max
No community added	DO (%)	76	32–130	81	51–127	81	43–151	77	46–138
	pH	8.19	6.97– 9.61	8.17	7.04–8.95	8.15	7.01–9.69	8.06	7.06–9.18
	EC (µS/cm^2)	548	462–707	531	427–648	537	455–678	550	392–679
	TDS (mg/liter)	367	273–469	353	268–431	359	257–447	368	281–456
	Chl (RFU)	5.9	0.9–20.3	4	0.7–10	4.8	0.5–21.2	3.6	0.8–17.6
Community added	DO (%)	95	50–154	106	62–166	100	58–154	120	62–187
	pH	8.17	6.61–9.56	8.22	6.49–9.34	8.29	6.63–9.30	8.71	6.85–9.77
	EC (µS/cm^2)	457	336–556	450	351–556	464	292–621	443	286–545
	TDS (mg/L)	306	220–400	301	217–392	312	216–417	294	195–385
	Chl (RFU)	0.6	0.3–1.9	0.8	0.4–5.5	0.8	0.2–6.5	0.6	0.3–1.7

Physicochemical parameters

Temperature was measured every two hours using a µS-LOG540 data logger (Driessen + Kern, Bad Bramstedt, Germany) in two randomly selected microcosms of each set-up type. The mean values (min-max) were 21.9° C (15.6–30° C) and 21.9° C (15.5–29.6° C) for the "no community added" and "community added" microcosms, respectively.

We measured the other abiotic parameters at least once after each application of thiacloprid. Dissolved oxygen (DO, in % saturation) was measured *in situ* with an electronic oxymeter ExStik DO600 (Walthman, MA, U.S.A.). Electrical conductivity (EC, µS/cm), pH, and total dissolved solids (TDS, mg/liter) of water were measured *in situ* using a multimeter ExStik II EC500 (Waltham, MA, U.S.A.). The chlorophyll was measured in relative fluorescence units (RFU) using a spectrofluorometer Spectramax Gemini EM (Sunnyvale, U.S.A.; wavelengths of 400 nm for excitation, 700 nm for emission, and 690 nm as cutoff). The water parameters DO, EC, pH, TDS, and chlorophyll did not correlate with any treatment-related effects (Pearson correlations, $p > 0.05$ in all relations).

Validation of exposure concentration

To quantify the actual thiacloprid concentrations over time, the microcosms were always sampled two hours after each of the three applications of thiacloprid and at least twice between two subsequent applications. A subsample of 500 ml of water was collected from each microcosm. The subsamples from the same exposure concentration were pooled to a single sample. Before measurements, samples were concentrated using solid phase extraction on a Chromabond C18 Hydra (Macherey-Nagel, Düren, Germany). Analyses were performed with liquid chromatography (high-performance liquid chromatography system with Diodenarray Detector II Series 2000, binary pump, autosampler, and column oven 30C; Perkin Elmer, Wellesley, MA, U.S.A.). The injection volume was 100 µl, dissolved in 25% acetonitrile/water solution with gradient-grade pump program.

Table 3. Median effective concentration (EC50) of thiacloprid for tested invertebrate taxa. Taxa were collected from uncontaminated "community" microcosms. Toxicity tests were conducted as Rapid tests (Kefford 2013). Post-exposure observation time ranges from 1 to 25 days. The 95% confidence intervals are given in parentheses.

Taxa	Time (days)	EC_{50} (µg/liter)
Cladocera	1 d	> 100,000
	4 d	8,615 (3,577 – 20,747)
	6 d	3,455 (1,759 – 6,784)
	9 d	1,654 (777 – 3,522)
Copepoda	1 d	> 10,000.00
	4 d	1,258 (538 – 2942)
	6 d	39 (15 – 101)
	9 d	2.51 (1.27 – 4.95)
Ostracoda	1 d	> 100.00
	4 d	84 (18 – 391)
	6 d	8.22 (4.69 – 13)
	11 d	6.74 (4.14 – 10)
	15 d	5.11 (3.51 – 7.53)
	18 d	4.64 (3.29 – 6.54)
	25 d	3.16 (NR)
Larvae *Cx. pipiens*	1 d	38 (31 – 45)
	2 d	31 (NR)
	3 d	28 (24 – 32)
	6 d	7.20 (5.17 – 45)
Larvae Chironomidae	1 d	> 1,000.00
	2 d	>1,000.00
	3 d	61 (3.95 – 969)
	6 d	0.25 (0.01 – 8.00)
Larvae Odonata	1 d	464 (212 – 1012)
	2 d	316 (159 – 628)
	6 d	158 (81 – 308)
	9 d	79 (38– 162)

NR: not reliable.

The limit of detection was 0.01 µg/liter. A LiChrospher 60, RP-select B, 5 µm column (Merck, Darmstadt, Germany) was used for separation (Kommunale Wasserwerke, Leipzig, Germany).

Data analyses

To identify significant differences between two samples, the nonparametric Wilcoxon rank sum test was applied. We assessed the community effect on oviposition and larval abundance of *Cx. pipiens* by comparing the differences in these parameters between the "no community" and "community" control microcosms. Because natural enemies generally have a negative influence on the abundance of mosquito larvae, we correlated the abundances of larval *Cx. pipiens* and their antagonists at the community level using linear regressions. Because of differences in sampling days between *Cx. pipiens* and antagonists, the abundance of *Cx. pipiens* was linearly interpolated. We also used linear regression to link the long-term sensitivity of taxa to thiacloprid at the 10 µg/liter concentration of the microcosm experiment and the six-day EC_{50} of the laboratory test. The Wilcoxon rank sum test was conducted in PASW version 17.0 (SPSS Inc. 2009), whereas linear regressions were conducted in GraphPad Prism version 5.00 for Windows. All figures were created in GraphPad Prism. For statistical analyses, abundance data were log-transformed. A p-value of 0.05 was used to define significance for all statistical analyses. Data for day 0 (starting point of the experiment) are not displayed for clarity.

RESULTS

Thiacloprid concentrations

Measured and nominal concentrations in the microcosms were within the same range (Figure 1). Peak concentrations of thiacloprid were detected in the water column of microcosms after each treatment and were on average 14% (± 6% standard error) higher than the nominal concentration. Twenty days after the last treatment, thiacloprid was reduced from the initial concentration by 32.6% in the "no community added" microcosms and by 66.6% in the "community added" microcosms.

Community effect on the population of *Cx. pipiens*

In the "added community" microcosms, oviposition and larval abundance of *Cx. pipiens* were strongly negatively affected. Control ponds generally had lower larval abundances and oviposition in the "community added" microcosms than in those of "no community added" (Figure 2; Wilcoxon rank sum test, $p < 0.05$).

Sensitivity of larvae of *Cx. pipiens* to thiacloprid at individual, population, and community levels

In laboratory toxicity tests, individual larvae of *Cx. pipiens* were sensitive to thiacloprid with a six-day EC_{50} of 7.20 µg/liter. A comparably higher sensitivity was observed for larvae of Chironomidae (six-day EC_{50} of 0.25 µg/liter), whereas comparably lower sensitivities were observed for antagonists both as competitors for food (six-day EC_{50} for Ostracoda, 8.22 µg/liter; six-day EC_{50} for Copepoda, 39 µg/liter; six-day EC_{50} for Cladocera, 3,455 µg/liter) and as predators (six-day EC_{50} for Copepoda, 39 µg/liter; six-day EC_{50} for Odonata larvae, 158 µg/liter; Table 3).

In microcosms without added community, populations of *Cx. pipiens* were not affected by the first exposure to thiacloprid at any concentration (Figure 3A). Additionally, we observed no significant effect of thiacloprid at 0.1 and 1 µg/liter over the complete time period. However, at the highest concentration of 10 µg/liter, we detected a temporal reduction in population abundance after the second treatment with thiacloprid (days 22-28; Wilcoxon rank sum test, 10 µg/liter vs pooled treatments of control and 0.1 and 1 µg/liter, $p = 0.018$) and the third treatment (days 36-43; Wilcoxon rank sum test, 10 µg/liter vs pooled treatments of control and 0.1 and 1 µg/liter, $p = 0.04$; Figure 3A). The reduced population abundance of *Cx. pipiens* observed after the second treatment was followed by a recovery two weeks later (Figure 3A).

In microcosms with added community, the abundance of *Cx. pipiens* populations decreased in the control and at all concentrations during the four weeks of observation (Figure 3B). Only after the second exposure to the highest treatment with 10 µg/liter thiacloprid, populations of *Cx. pipiens* were temporarily positively affected as shown by an increase in larval abundance

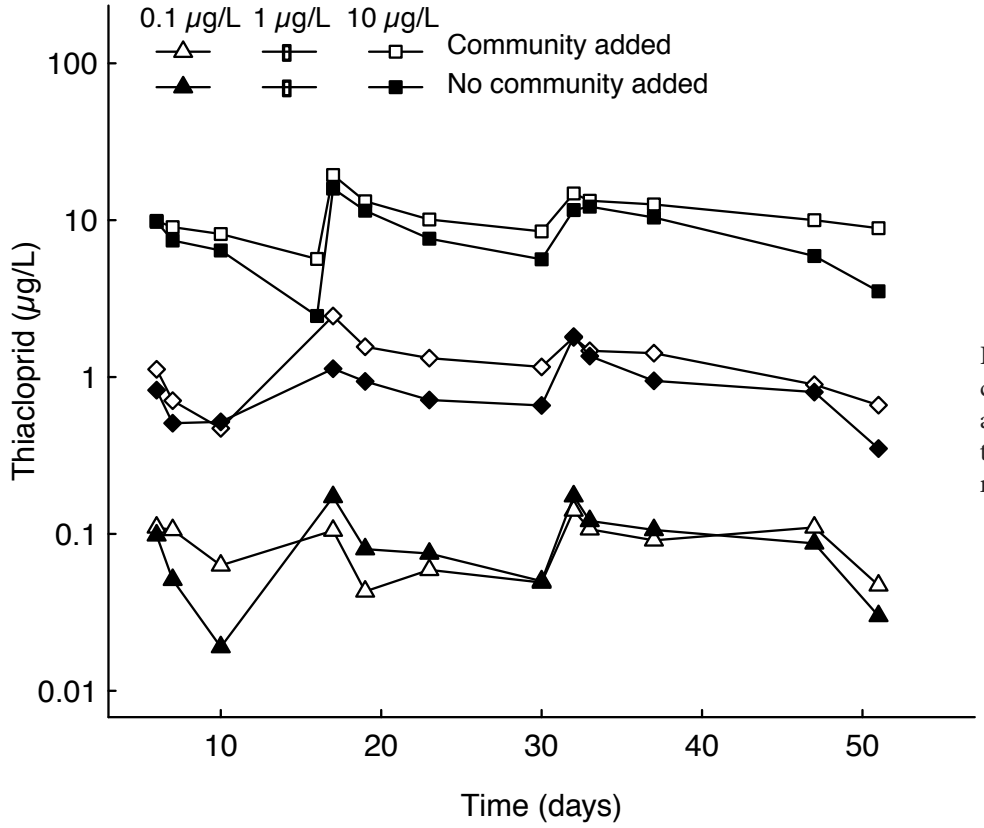

Figure 1. Thiacloprid degradation in "no community added" and "community added" microcosms. Arrows indicate the first, second, and third exposures of microcosms to thiacloprid.

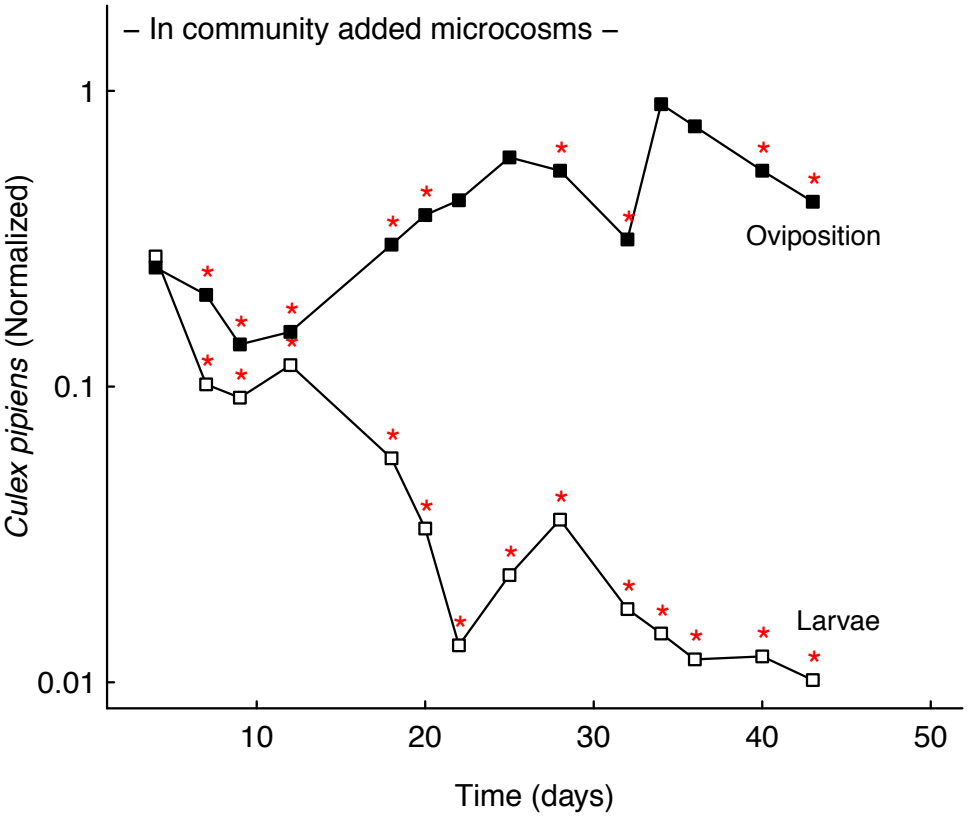

Figure 2. Oviposition and larval abundance of *Cx. pipiens* in the "community added" microcosms in relation to those of "no community added." Only control ponds are considered. Asterisks indicate a significant difference ($p < 0.05$, non-parametric Wilcoxon rank sum test).

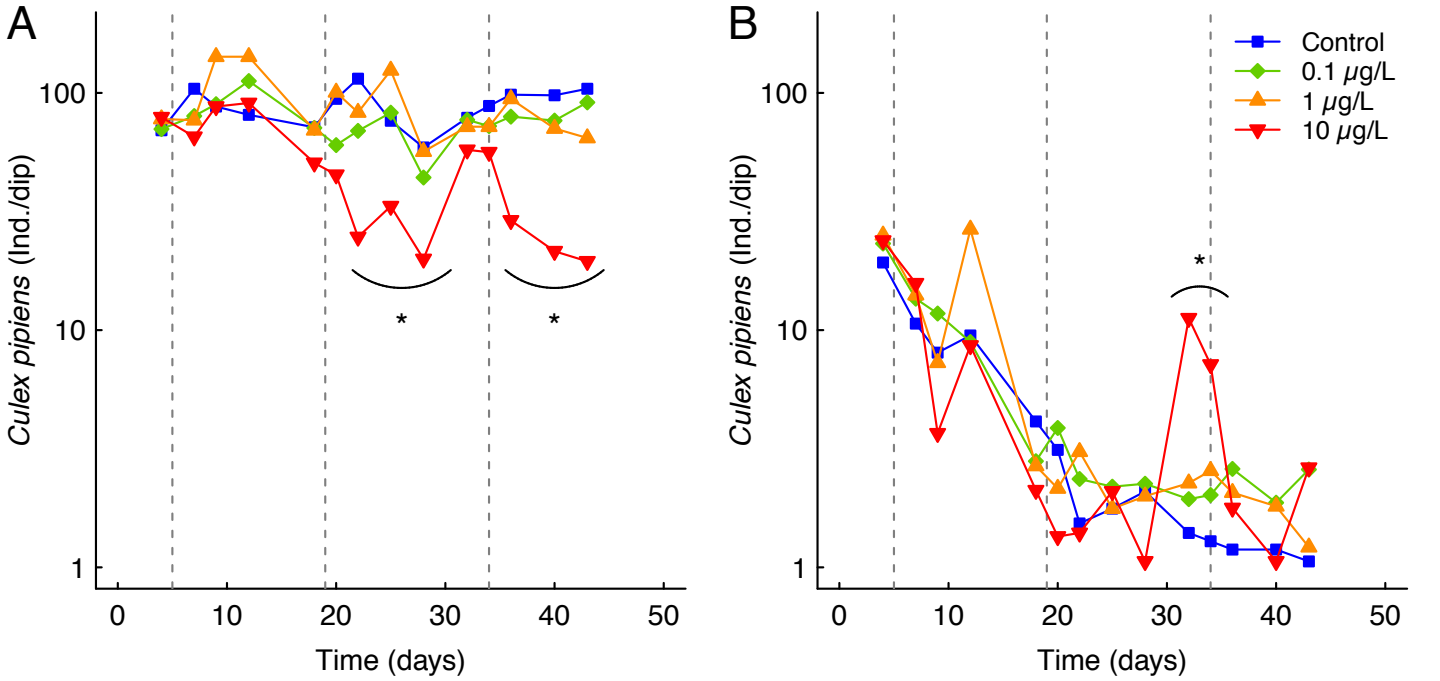

Figure 3. Time course of *Cx. pipiens* larvae in "no community added" and "community added" microcosms. The vertical dashed lines indicate the first, second, and third exposures of microcosms to thiacloprid. For the comparison of means, a Wilcoxon rank sum test was applied (10 µg/liter vs pooled treatments of control and 0.1 and 1 µg/liter). Asterisks "*" and "**" represent significant differences at $p < 0.5$ and $p < 0.01$, respectively.

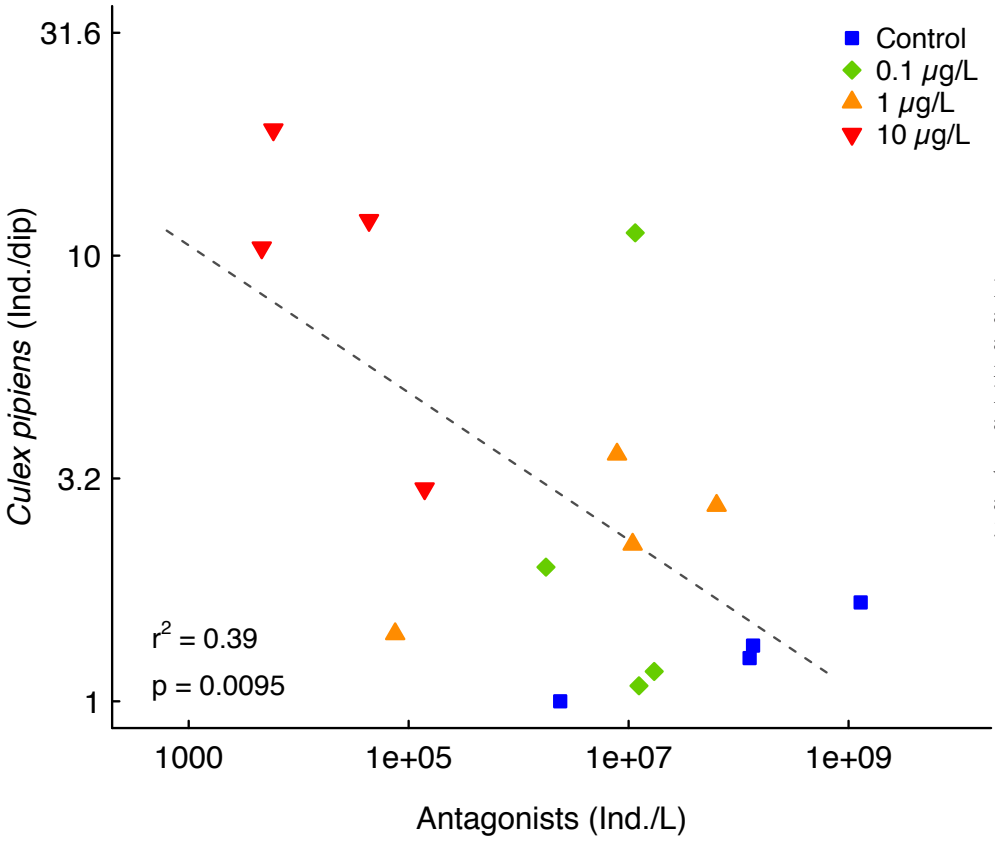

Figure 4. Relationship between the abundance of *Cx. pipiens* and associated antagonists in the "community added" microcosms at approximately day 33. Day 33 represents the time point of peak abundance of *Cx. pipiens* after exposure to 10 µg/liter. The abundance of *Cx. pipiens* was quantified as the average of days 32 and 34. Antagonists were sampled at day 35.

from day 32 to 34 at 10 µg/liter (Figure 3B; Wilcoxon rank sum test, 10 µg/liter vs pooled treatments of control and 0.1 and 1 µg/liter, $p = 0.002$).

Regression analysis revealed that the peak abundance of *Cx. pipiens* larvae was negatively correlated with the abundance of antagonists (Figure 4; linear regression, $r^2 = 0.39$, $p < 0.01$). Antagonists were defined as competitors for food (i.e., Cladocera, Copepoda, Ostracoda, and larvae of Chironomidae) and predators (Copepoda and larvae of Odonata) on day 35.

To understand the underlying biotic interactions, the development and long-term sensitivity of all taxa in the 10 µg/liter treatment were also analyzed (Figure 5). The 48-day development of taxa after exposure to 10 µg/liter separated the community into long-term insensitive and sensitive species. The abundance of long-term insensitive species was unaffected at day 48, with these species included in the orders Cladocera, Odonata, and Chironomidae. The abundance of long-term sensitive species decreased over the experiment to day 48, which was observed for species in the orders Copepoda and Ostracoda (Wilcoxon rank sum test, $p = 0.029$, for both taxa; Figure 5). This long-term sensitivity of populations in the microcosms on day 48 was also compared with the short-term sensitivity of individuals in laboratory toxicity tests (6-day EC_{50}; Figure 6). In general, the long-term sensitivity of populations and the short-term sensitivity of individuals were positively and significantly linked for the following organisms: Cladocera, Copepoda, Ostracoda, and larvae of Odonata. However, two taxa did not follow this trend, the larvae of *Cx. pipiens* and Chironomidae. Indeed, significant variation was observed only when data of these two last taxa were omitted from the linear regression (Figure 6; linear regression without *Cx. pipiens* and Chironomidae, $r^2 = 0.96$, $p = 0.019$).

DISCUSSION

Concentration of thiacloprid

In the microcosm experiment at day 53, which corresponded to 20 days following the last treatment, thiacloprid was detected in the water at approximately one-third of the initial concentration in "community added" microcosms. By contrast, in a stream mesocosm system, Beketov et al. (2008) noted a more rapid degradation of thiacloprid and found no detectable amount of thiacloprid in the water column 13 days following the application of an initial concentration of 2.83 µg/liter (detection limit < 0.01 µg/liter). The more light-exposed lotic ecosystem that contained only a 25 cm water level might explain the faster degradation rate of thiacloprid in that study. Indeed, Flores-Céspedes et al. (2012) reported faster degradation of imidacloprid, another neonicotinoid insecticide, when exposed to an increase in light intensity.

Sensitivity of *Cx. pipiens* larvae to thiacloprid at individual, population, and community levels

At the individual level, the six-day EC_{50} of 7.2 µg/liter for *Cx. pipiens* is consistent with a published 14-day LC_{50} of 6 µg/liter (Beketov and Liess 2008a). Based on the known sensitivity of *Cx. pipiens*, we expected stronger effects of thiacloprid in the microcosms at the highest concentration of 10 µg/liter. However, without added community, the first treatment of *Cx. pipiens* larvae with 10 µg/liter thiacloprid had no observed effect in the microcosm experiment. Additionally, the repeated treatment with 10 µg/liter resulted only in a temporal and short-term reduction in the abundance of *Cx. pipiens* larvae, most likely a consequence of increased thiacloprid concentrations after the second and third treatments. We explained this low effect of thiacloprid at the population level by the high potential of *Cx. pipiens* to recover; one adult mosquito can lay several hundred eggs at a single time on the water surface of breeding sites (Becker et al. 2003). Additionally, oviposition strongly increases when low densities of conspecifics are in the water (Duquesne et al. 2011). Hence, at the concentration of 10 µg/liter thiacloprid, we argue that oviposition partly compensated for mortality due to pesticide exposure. These results are consistent with those identified by Liess et al. (2013), who found that *Cx. pipiens* was not affected even after six treatments of thiacloprid in the absence of competitors.

At the community level, a repeated exposure to 10 µg/liter thiacloprid decreased the abundances of antagonists and temporarily increased the abundance of *Cx. pipiens* larvae. We propose the following indirect mechanism for this positive effect on *Cx. pipiens* larvae. Thiacloprid acutely affected both *Cx. pipiens* larvae and antagonists; however, mosquitoes might have recovered more quickly than their antagonists because of (i) newly deposited eggs by unexposed adult females from the surroundings and control microcosms and (ii) a generally rapid larval development of mosquitoes (Duquesne and Liess 2010, Duquesne et al. 2011, Grigarick et al. 1990, Meyabeme Elono et al. 2016). Therefore, we inferred that species with a strong ability for recovery might show a reduced vulnerability to toxicants within the community context, which is consistent with the observation of Liess and von der Ohe (2005). These authors show a low vulnerability to pesticides for those species with a high recolonization potential and a high vulnerability for species with a low recolonization potential.

However, from the laboratory toxicity tests of the present study, we know that the two dipteran taxa, *Cx. pipiens* and Chironomidae, were more sensitive to thiacloproid than ostracods, copepods, cladocerans, and larvae of Odonata. In general, individual level toxicity information is used as a proxy for ecosystem sensitivity (Fleeger et al. 2003). An example is the lower tier assessment of the EU pesticide directive 2009/128/EC that extrapolates information on acute toxicity to field communities. We showed that the sensitivity of invertebrate taxa to thiacloprid at the individual level was generally positively correlated with their response at the community level. However, *Cx. pipiens* and Chironomidae did not follow this trend, because they had high sensitivity at the individual level but low vulnerability at the community level. Both species share an ecological trait, which is a high potential of recovery from adults outside the water (Becker et al. 2003). In our study, adults from other microcosms and surrounding areas might explain the increased recovery potential of *Cx. pipiens*.

We conclude that the physiological sensitivity of a species is not sufficient to predict a toxicant effect at the ecosystem level; other traits such as recovery potential are also highly relevant. This was also shown when linking pesticide exposure with trait distributions of invertebrate species in an agricultural landscape (Liess and Von Der Ohe 2005). Here, we showed that the physiological sensitivity and the recovery potential contributed approximately equally to explain the link between sensitive species

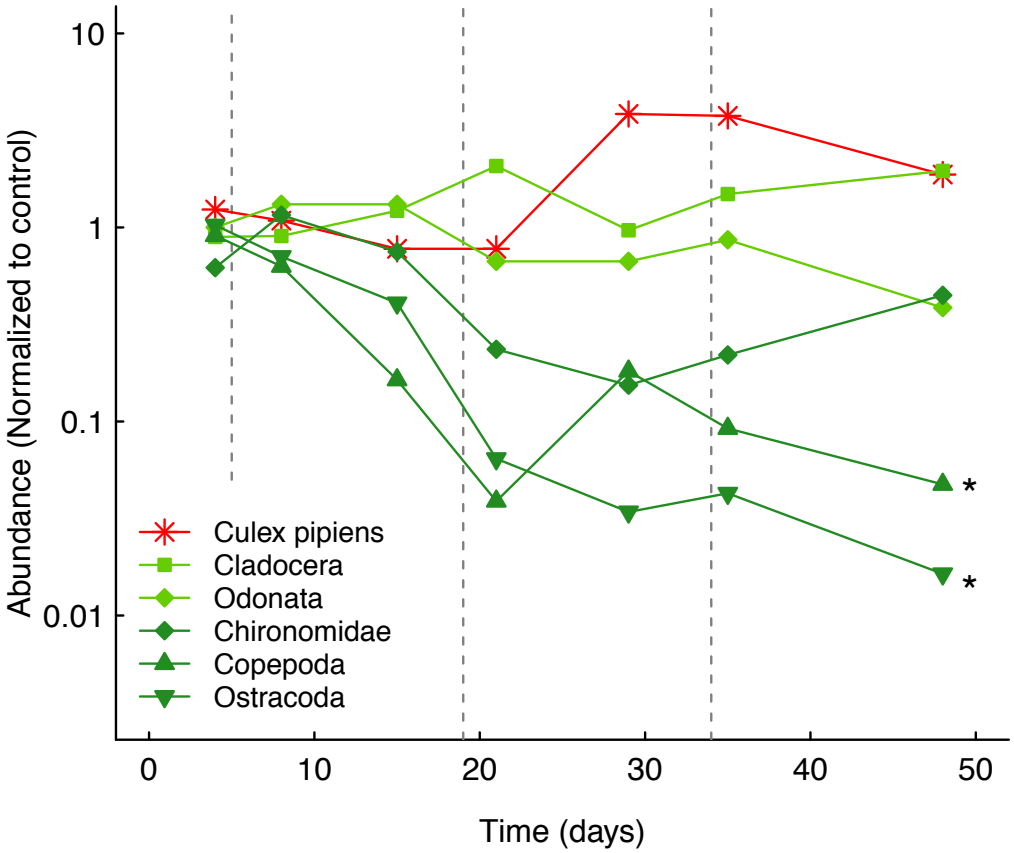

Figure 5. Time course of the abundance of taxa in the "community added" microcosms with repeated exposure to 10 µg/liter thiacloprid. The abundance of taxa is normalized to the control. The normalized abundances of Copepoda and Ostracoda were significantly lower than those in the control. Asterisks represent significant differences from the control (Wilcoxon rank sum test). The vertical dashed lines indicate the first, second, and third exposures of microcosms to thiacloprid.

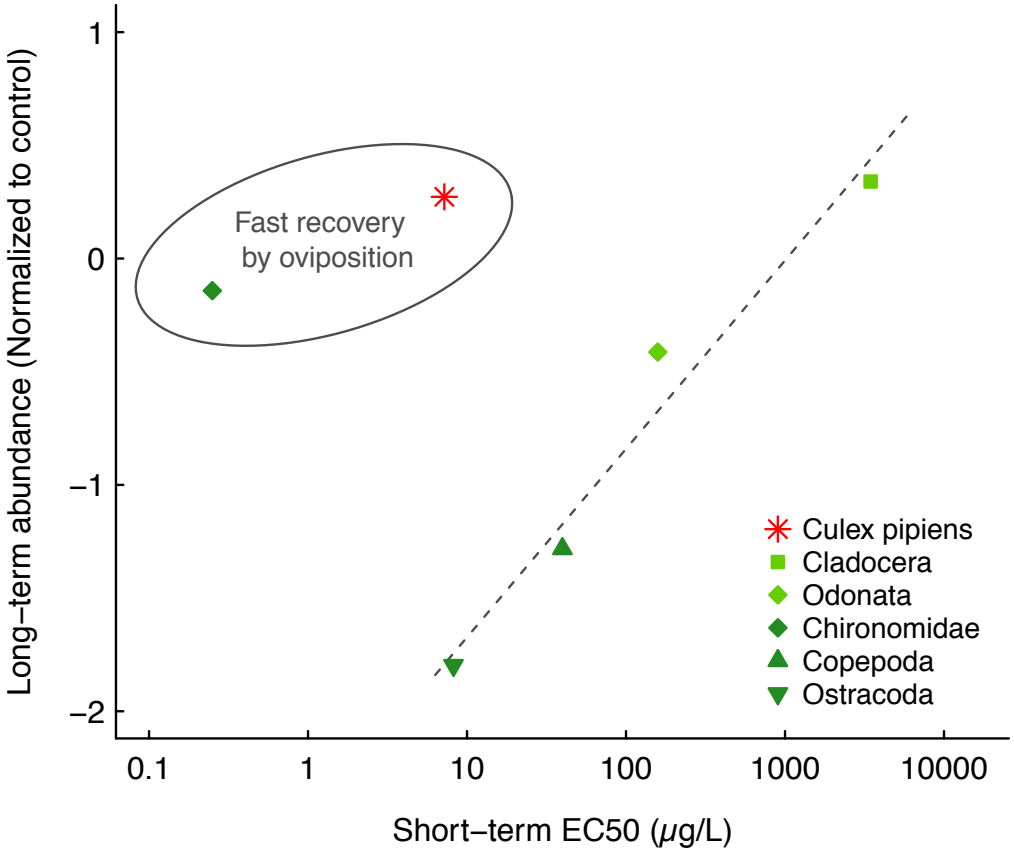

Figure 6. Relationship between short-term effects in the laboratory and long-term effects in the microcosms. Short-term effects in the laboratory were quantified as the six-day EC_{50} by Rapid testing (Kefford 2013). Long-term effects in the microcosms are given as final taxa abundance in the "community added" microcosms that were repeatedly treated with 10 µg/liter thiacloprid (see Figure 5). Taxa abundances for long-term effects are normalized to the control. Except for larvae of *Cx. pipiens* and Chironomidae (encircled), the final normalized abundance of the other taxa at the community level was positively linked to the respective short-term EC_{50} at the individual level.

(Liess and Von der Ohe 2005) and toxicant stress.

Because of high potential to recover from pesticide stress, *Cx. pipiens* larval populations are likely to recolonize water bodies after pesticide treatment when antagonists are negatively affected. In the current study, antagonists showed better potential for controlling the abundance of larval mosquitoes than that of the neonicotinoid insecticide thiacloprid. Therefore, mosquito control measures must ensure that natural competitors and predators are not affected; indeed, by contrast, these species should be used to increase the efficiency of these measures, as described within a patent for sustainable control (Liess and Duquesne 2014).

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