“Effect assessment and regulation of pesticide mixtures in aquatic ecosystems”

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Executive Summary

Water pollution is a major threat to biological diversity worldwide. Increased pesticide use, and their application as mixtures, is one of many drivers affecting habitat health. Most data used in risk assessment of pesticides are based on single species tests using single substances, at concentrations that are usually not environmentally realistic, with few assessments including sublethal endpoints.

In order to bridge the gap between laboratory toxicity testing using individual chemicals, and the effects of mixture toxicity on aquatic ecosystems, this study is an extension of a previous laboratory-based study (DPR contract # 10-C0096) where 10-day toxicity assessments of single and combined exposures of three commonly used insecticides were conducted. The effects of two pyrethroids; lambda-cyhalothrin and permethrin, and one organophosphate; chlorpyrifos, applied individually and in mixtures on *Chironomus dilutus* and *Hyalella azteca* were investigated. Lethal concentrations were then applied within a 6-month multi-species field study using mesocosms, composed of naturally developed invertebrate communities. The effects of a series of applications of tertiary contaminant mixtures resulted in significant decrease of *H. azteca* population, and the zooplankton species copepoda and cladocera. The lethal concentrations determined in single-species tests in the laboratory did not necessarily reflect the effects predicted in the environment. By using lab-based toxicity tests it is possible to determine ecologically relevant sublethal effects under controlled conditions within a very short period of time. Mesocosms on the other hand allow us to evaluate long-term community and food-web effects. Both approaches provide essential information for understanding mixture toxicity and evaluating their effects on aquatic ecosystems, which can be used in risk-assessments of contaminants of concern.

1. Background and goals

Aquatic ecosystems and food webs in the Sacramento-San Joaquin (SSJ) River Delta are frequently adjacent to areas of intense pesticide use that may discharge complex mixtures of contaminants into surface waters. The protection goals of legislation and regulatory authorities include populations, communities, and ecosystems, in addition to individuals. Thus more inclusive studies are necessary to ensure that toxicological assessments are effective in aiding ecosystem management efforts. Invertebrates are of special interest not only because they share biochemical pathways with humans, but also because they sustain fisheries.

Our **goals** were to conduct a detailed assessment of the impact of two pyrethroids, lambda-cyhalothrin and permethrin, and the organophosphate, chlorpyrifos, on invertebrate communities, both macroinvertebrates and zooplankton, including monitoring the chemical fate of the three chemicals in both the water column and sediment.

Our specific **objectives** were thus:

- To determine long-term contaminant mixture effects on macroinvertebrate community structure, function, and biomass, encompassing different life stages of aquatic invertebrates and their seasonal development.
- To monitor the fate of contaminants, both in the water column and sediment, and to assess how this passage affects the species living in the different habitats.

2. Material and Methods

2.1 Test system and colonization phase

The mesocosm system used for this study was constructed at the UC Davis Putah Creek Riparian Reserve. It consists of 16 solitary PVC tanks. Each tank was filled with a 10cm layer of a clean sand-sediment mixture consisting of 50% sand and 50% natural sediment. Tanks were filled with approximately 1,330 L of a mixture of clean well water and uncontaminated pond water from a pond close to the study site. Lack of biologically significant contamination was confirmed by conducting a 96h toxicity test using the sensitive amphipod species, *Hyalella azteca,* provided by the Aquatic Health Program at UC Davis. Aquatic plants were evenly added to each tank, which consisted of the two submerged species (*Elodea* sp. and *Myriophyllum* sp.); these were obtained from Putah Creek at the Riparian Reserve. When the mesocosms were set up, functional groups of invertebrates relevant to the fish species in the Delta were added and/or colonized naturally. These include, but were not limited to copepods, cladocerans, amphipods, and chironomids. Immigration of flying aquatic insects from Putah Creek and adjacent ponds promoted the development of an intact community, and supported recovery by recolonization following

pesticide application. Because we were especially interested in the effects on the amphipod species *Hyalella azteca* and the cladoceran *Daphnia magna* we added a number of animals to each tank from an in-house culture from the Aquatic Health Program, UC Davis. Two 5 gallon buckets were filled with water from this in-house culture containing a high density of *H. azteca* and *D. magna*, respectively. The content was stirred to achieve an equal distribution of animals in the bucket, and 500ml of the culture were transferred to each mesocosm tank.

2.2 Biological sampling

Sampling took place weekly from six weeks (day -41) before the first pesticide application (day 0) and four months past the first application (final sampling day = day 134). Physicochemical parameters of each tank such as dissolved oxygen, percentage of oxygen, electronic conductivity, specific conductivity, pH, and temperature were measured in the morning of each sampling day.

For zooplankton identification we collected one sample consisting of four sub-samples per mesocosm tank. By using a PVC tube (4cm in diameter and 1m in length) sub-samples were taken from each tank. Total water volume is calculated by noting the depth of the sampled water column. Water was poured through a stainless steel sieve (pore size = 63µm) to remove metazoans and zooplankton were collected from four sub-samples and the water returned to the corresponding tank. Animals in the sieve were transferred to Polyethylene-bottles and fixed with the staining solution Rose Bengal.

For macroinvertebrate identification we used the following sampling methods: 1) Using a sampling mesh (pore size = 125 µm) samples were taken in form of three sweeps (two along the sides of the tank walls and one through the free water). 2) Benthic invertebrates were sampled using purpose-built habitat samplers. 3) In order to collect emerging invertebrates, floating emergence traps were positioned in each tank and sampled every 4-5 days. All organisms were counted and identified to the lowest practical taxonomic level on-site.

2.3 Pesticide Application

To model realistic application scenarios, commercial pesticide formulations were used for this study (Table **2**). Pesticide treatments and controls were randomly assigned to the tanks (Figure 1). There were five application events. Treatment 1 involved simply repeating applications of environmentally relevant concentrations derived from averaged values derived from the CEDEN database for the years 2011 and 2012 from monitoring studies, excluding runoff events, for each contaminant (database accessed on 061213). Application levels for Treatments 2 and 3 were based on laboratory assays and increased after the first and third applications as outlined in Table 2 (Hasenbein and Lawler 2013). Treatment 2 was based on concentrations lethal to *H. azteca* and Treatment 3 on lethality to *C. dilutus.* Mixtures were

formulated for an estimated combined toxicity of LC 10 for treatment 1, LC 25 for treatments 2 and 3, and LC 50 for treatments 4 and 5 (
Table **3**). Each spray event consisted of three different pesticide mixture concentrations, each applied to four tanks (Table 4). Pesticide mixtures were prepared in 250ml volumetric flasks, from which 50ml were evenly sprayed over each tank using commercially available 3L-pump sprayer bottles.

2.4 Analytical chemistry

Samples of both water and sediment were taken weekly following the first pesticide analysis from each tank (control and treated tanks). They were analyzed to trace the presence of the pesticides and common breakdown products in each mesocosm. Control tank samples were analyzed to make sure no pesticides entered these tanks when applying the pesticides to the treated tanks.

Water subsamples were collected using amber pre-labeled and kilned glass bottles (950 mL). Sediment subsamples were collected from the top 2 cm using pre-cleaned stainless steel spoons and carefully transferred to 950-ml amber pre-labeled and kilned glass bottles. All samples were transported on wet ice to the laboratory, stored in the dark at 4°C and extracted within two days of collection.

The surrogate trans-permethrin D6 (EQ Laboratories, Atlanta, GA) was added to each water sample before extracting using conditioned 6-ml solid phase-extraction C_{18} cartridges (Supelclean™ 500 mg, Sigma-Aldrich) at a slow drip under vacuum. To elute pesticides columns were rinsed twice with a 5-ml volume of a solution of hexane:ethyl acetate (1:1, v/v). Solvent elution (10 ml) from each column was collected and concentrated to 0.4 ml under a gentle stream of nitrogen.

Sediment samples were dried at 70ºC and ground using mortar and pestle. An aliquot of 10g of each sample was used for further analysis. After adding the surrogate Trans-Permethrin D6 (EQ Laboratories, Atlanta, GA) and 20 ml of a solution of hexane:dimchlormethane (3:7, v/v) dried sediment was sonicated for 30 min and centrifugated for 5 min. The solvent layer was transferred into another centrifuge tube, and the sediment extraction was repeated two times by adding 10 ml of a solution of hexane:dimchlormethane (3:7, v/v) each time, resulting in a total of 40 ml of added solution. All three solvent layers were collected and combined. The combined extracts were concentrated to 0.4 ml under a gentle stream of nitrogen and added on a preconditioned GCB/PSA cartridge (Supelclean™ ENVI-Carb™ II) at a slow drip under vacuum. The GCB/PSA cartridges were used to effectively remove plant pigments such as chlorophyll, and plant sterols from the final extracts without the loss of planar compounds. To elute pesticides, columns were rinsed with a 7-ml volume of a solution of hexane:dimchlormethane (3:7, v/v). Solvent elution (7 ml) were collected and concentrated to 0.4 ml under a gentle stream of nitrogen.
The internal standard Dibromooctafluorobiphenyl (Chem Service, West Chester, PA) was added to the concentrated extracts prior to GC analysis.

All final extracts were analyzed using gas chromatography negative chemical ionization mass spectrometry (GC-NCI-MS) on Agilent 5973 series gas chromatograph (Agilent Technologies, Palo Alto, CA), equipped with a split-splitless injector (280°C, splitless, 1.5-minute purge time). The column was a Supelco DB-5MS column (30 m x 0.25 mm with a 0.3 µm film thickness). Instrumental calibration was performed using nine sets of calibration standard solutions with each pesticide (Chem Service, West Chester, PA), the surrogate trans-permethrin D6 (EQ Laboratories, Atlanta, GA), and the internal standard dibromooctafluorobiphenyl in hexane. Quantification will be based on peak area using the standards.

2.5 Statistical Analysis

The collected data were managed in database "Access 2010" for Microsoft and then further processed in "Excel 2010" and "R", version 3.0.3 (R Core Team 2014). To test normality of the data Shapiro-Wilk test was used. For testing homogeneity of variances the Levene-test was carried out. When data was distributed normally and homogeneity of variances was not given, the Dunnett's test was carried out to test the significance of treated tanks compared to the control treatments.

Abundance of each taxon was plotted against time. Ranked species abundance are a manner of graphically presenting patterns of relative species abundances. They are based on the ranking of species (or higher taxa) in decreasing order of their importance in terms of abundance. The ranked abundances are expressed as percentage of the total abundance of all species. Macroinvertebrate data presented herein represent the sum of the species numbers from netting and habitat samplers.

Multivariate statistics will be added to the peer-reviewed manuscript issuing from this work.

3. Results and Discussion

3.1 Physical Parameters

At the beginning of the study the temperature ranged between 21 and 17°C until day 36, followed by a steady decline until final sampling day (8°C) due to seasonal changes in temperature (Figure 2). Oxygen concentrations varied between 3.3 (minimum value in the control treatment on day 77) and 14.8 mg/L (maximum value in control treatment on day 99) across all treatments and sampling days (Figure 3). Contaminant application did not affect oxygen levels (average oxygen concentration of 7.4 mg/L, SD = 1.46, in both control and treated tanks). The pH was stable during the entire study period in all tanks (9.6, SD = 0.3), (Figure 4). The electroconductivity represents the number of ions dissolved in the water. This value varied between 220 and 1279 µS/cm across all treatments and over the entire study period (Figure 5). These fluctuations may be due to the water refilling that regularly was conducted due to the evaporation of the tank water. Since all treated tanks remained within control values, the fluctuations were not due pesticide exposure.

Homogeneity in the physical parameters confirms the successful establishment of the mesocosm system. All measured parameters play an important role in aquatic ecosystems. Oxygen levels, for example, are an integrative parameter which mainly depend on the balance between production (i.e. photosynthesis) and consumption (i.e. respiration) rates (Caquet *et al.* 2001). The temperature of an aquatic system plays an important role for the intensity of pesticide toxicity. Harwood *et al.* (2009) investigated that a temperature decrease of 10°C increased the toxicity of pyrethroids but decreased the toxicity of chlorpyrifos.

3.2 Biological sampling

A total of 23 macroinvertebrate taxa were found in both the controls and the treatments (Table 5). The suborder Zygoptera (damselflies) and the amphipod *Hyalella azteca* were the two most affected groups of macroinvertebrates. Following the second pesticide application there was a non-significant trend toward a decrease in *H. azteca* abundance in T1 and T3. However, following the fourth application, *H. azteca* were less abundant in T1 and T3 on four consecutive days (day 113, 120, 127, 134) (Dunnett's test, $p < 0.05$). There was no significant difference measurable for T2 (Figure 6). Zygoptera showed decreases in treated tanks on days 113 and 127 in T3 compared to the control treatments (Figure 7). No dead animals were collected or counted in these treatments, indicating that insects close to metamorphosis might have left the system due to differing development times rather than being affected by the pesticide applications. The addition of control treatments and exposing them to the same environmental conditions ensures that observed effects were due to pesticide applications.

Emergence traps catches did not result in any significant differences between treated and control tanks due to the low number of insects collected (no graphs shown herein). A more specified approach will be developed for future studies investigating effects of insecticides.

A total of 18 zooplankton taxa were found in this study (Table 6). The species *Daphnia magna* was the most sensitive zooplankton species in this study (Figure **8**). Following the third application (day 92) abundance in T3 significantly decreased down to an average of 10 individuals/L (SD = 0.59) compared to the control (645 individuals/L, SD = 64.48). Except for day 99 when only a strong trend was present, the abundance of *D. magna* remained significantly lower until the last day of the study. Abundance in T2 was significantly decreased between day 106 and 120, but was within control range from day 127 on. Abundance in T1 was significantly decreased on two non-consecutive sampling days (day 106 and 120) which also represented the days following pesticide application.

The subclass Copepoda was the second most sensitive zooplankton group, represented by the order cyclopoida. On Day 36 all three treatments had lower copepod populations than controls, and T3 was also lower on days 92, 120, 127, and 134.

T2 was the only treatment that showed very little toxic effects and it also had the lowest amount of lambda-cyhalothrin, but it contained more of the other two toxins than T1, the second-lowest treatment. This potentially indicates that lambda-cyhalothrin is the driving force for toxicity in the mixtures.

3.3 Analytical Chemistry

No pesticides were detected in the control tanks over the entire study period. The nominal concentrations for all applications were confirmed through pesticide measurements of the water samples. In all three treatments chlorpyrifos concentration increased over time in the water column, whereas pyrethroids dissipated from the water column within a few weeks (Figures 10-12). For the pesticide analysis in the sediment concentrations were much lower than in the water column, with a peaks of the pesticides following applications (Figures 13-15).The main reason for this is that pyrethroids are highly nonpolar chemicals of low water solubility and high octanol-water partition coefficients resulting in a high affinity to any type of surface (Wheelock *et al.* 2005). Laskowski (2002) summarized physical and chemical environmental properties of pyrethroids confirming that lambda-cyhalothrin and permethrin have very high log K_{ow} values with 7.00 and 6.10, respectively. The increase of chlorpyrifos on the water column is likely due to its less hydrophobic nature (log K_{ow} = 4.7).

4. Conclusion

This study provided long-term information on both biological responses and pesticide fate following a set of five pesticide applications over the course of six months. Only few species were affected by the pesticide application indicating that in future studies focus should be put on certain key-species or species of interest. This will also ensure to keep the workload of an extensive study such as this in an acceptable range. Copepods and *Daphnia magna* were among the most severely affected organisms in the test system, and more focus should be put on them in future studies. The use of the emergence traps needs to be developed in more detail for future studies. The lethal concentrations determined in single-species tests in the laboratory did not necessarily reflect the effects predicted in the environment. By using lab-based toxicity tests it is possible to determine ecologically relevant sublethal effects under controlled conditions within a very short period of time. Mesocosms allow us to evaluate long-term community and food-web effects. Both approaches provide essential information for understanding mixture toxicity and evaluating their effects on aquatic ecosystems, which can be used in risk-assessments of contaminants of concern. For future studies a combined application of herbicides and insecticides will allow to further investigate realistic exposures of aquatic ecosystems.

5. Deliverables

5.1 Publications

1) A comparison of the sublethal and lethal toxicity of four pesticides in *Hyalella azteca* and *Chironomus dilutus* (submitted to Environmental Science and Pollution Research)

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Abstract

Laboratory toxicity testing is the primary tool used for surface water environmental risk assessment, however there are critical information gaps regarding the sublethal effects of pesticides. Sublethal toxicity can negatively affect organism fitness, and often occurs at fractions of lethal effect concentrations. In this study, we compared the lethal and sublethal toxicities of four commonly used pesticides, bifenthrin, permethrin, cyfluthrin, and chlorpyrifos, on two freshwater invertebrates, *Chironomus dilutus* and *Hyalella azteca*. We analyzed lethal toxicity over ten day (10d) exposures, and measured the sublethal toxicity on swimming motility and growth of surviving individuals at the end of each 10d test. *H. azteca* were between 4 and 100 times more sensitive than *C. dilutus* to the pesticides tested. Pyrethroids were more toxic than the organophosphate chlorpyrifos in both species, and bifenthrin and cyfluthrin were the most potent. Growth was a good indicator of toxicity for *C. dilutus* with cyfluthrin being the most toxic, followed by permethrin, and bifenthrin. Motility served as the best endpoint in assessing sublethal effects in both species. Decreased motility was detected at concentrations as low as 10% of the corresponding LC_{50} values; levels commonly detected in environmental water samples. Growth of *H. azteca* was significantly affected by bifenthrin only. Invertebrates like *C. dilutus* and *H. azteca* are important components of the aquatic food web, and lethal and sublethal effects elicited by pesticide exposures have the potential to disrupt food webs and community structure in aquatic environments.

2) Standard toxicity assessments can underestimate the effects of environmentally relevant pesticide mixtures upon aquatic organisms (in preparation)

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Abstract

Aquatic communities are often subject to complex mixtures of contaminants. Mixture exposures even at low levels can result in additive, synergistic, or antagonistic effects. We investigated the tertiary mixture effects of type I (permethrin) and type II (lambda-cyhalothrin) pyrethroids, and an organophosphate (chlorpyrifos) on the sublethal endpoints growth and swimming mobility of *Chironomus dilutus,* and *Hyalella azteca* following 10-day-exposures. Median lethal concentrations (LC50) were used for each compound. *C. dilutus* growth was inhibited following exposure to pesticide mixtures below 1/8 of LC50, to lambda-cyhalothrin applied singly at concentrations greater than 1/4 of LC50, and to permethrin in dosages greater than 1/6 of LC50. Decreased mobility resulted from mixture concentrations greater than 1.5 times of LC50 (*C. dilutus*) or greater than 1/8 of LC50 (*H. azteca*), and exposure of each species to all single pesticides at concentrations greater than 1/4 of LC50. Our data suggest that ecologically important sublethal effects of insecticide mixtures can occur at concentrations that are 8-fold lower than the corresponding LC50 values. Using sublethal endpoints in ambient water monitoring efforts can indicate the presence of low-levels of contaminants in water or sediment samples, at concentrations below the limit of detection of current-use analytical methods.

3) Pesticide mixture toxicity assessments differ between single species tests and mesocosm studies (in preparation)

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Abstract

Water pollution is a major threat to biological diversity worldwide. Increased pesticide use, and their application as mixtures, is one of many drivers affecting habitat health. Most data used in risk assessment of pesticides are based on single species tests using single substances, at concentrations that are usually not environmentally realistic, with few assessments including sublethal endpoints.

In order to bridge the gap between laboratory toxicity tests using individual chemicals, and the effects of mixture toxicity on aquatic ecosystems, we first conducted 10-day toxicity assessments of single and combined exposures of three commonly used insecticides: two pyrethroids; lambda-cyhalothrin and permethrin, and one organophosphate; chlorpyrifos, on lethal and sublethal effects on *Chironomus dilutus* and *Hyalella azteca*, two important ecotoxicological testing organisms. We then evaluated these conditions on community composition within a 6-month multi-species field study using mesocosms, composed of naturally developed invertebrate communities.

In the laboratory-based single-species tests, growth and motility were significantly affected at ecologically relevant concentrations. The effects of mixture exposures, at relative toxic concentrations, were less severe than those observed in the single exposures. In the mesocosms, the effects of a series of applications of tertiary contaminant mixtures resulted in significant decrease of *H. azteca* population, and zooplankton species such as copepods and cladocera. The lethal concentrations determined in single-species tests in the laboratory do not necessarily reflect the effects predicted in the environment. By using lab-based toxicity tests it is possible to determine ecologically relevant sublethal effects under controlled conditions within a very short period of time. Mesocosms on the other hand allow us to evaluate long-term community and food-web effects. Both approaches provide essential information for understanding mixture toxicity and evaluating their effects on aquatic ecosystems, which can be used in risk-assessments of contaminants of concern.

5.2 Presentations

1) SETAC North America Annual Meeting, Nashville, TN, November 17-21, 2013, Poster presentation: A long-term effect assessment of tertiary pesticide mixtures on aquatic invertebrate communities using mesocosms

2) Interagency Ecology Meeting, Lake Natoma, CA, February 26-28, 2014, Oral presentation (invited): Mesocosoms: A Tool to Assess Long-term Efffects of Pesticide Mixtures on Aquatic Invertebrate Communities

3) PREP PESTICIDES & WATER QUALITY: URBAN-RURAL IMPACTS COURSE, Davis, CA, April 10, 2014, Oral presentation (invited): UCD/CDPR study: Assessing the complex effects of pesticide mixtures on aquatic communities

4) NorCal SETAC Annual Meeting, Berkeley, CA, April 6, 2014, Oral presentation: The effects of pesticide mixtures on aquatic invertebrate communities – a mesocosm study

Acknowledgments

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Tables

Table 1 Sampling Schedule for the entire study period. Grey highlighted dates represent the five application days, no biological sampling was conducted on those days, only water and sediment samples were taken for pesticide analysis. Day 0 = day of first application.

Date	May 09	May 16	May 23	May 30	June 06	June 13	June 19	June 21	June 27	July 04	July 11	July 18	July 25	Aug 01	Aug 08	Aug 09
Day after 1st application	-41	-34	-27	-20	-13	-6	0	2	8	15	22	29	36	43	50	51

Date	Aug 15	Aug 22	Aug 29	Sept 04	Sept 12	Sept 17	Sept 19	Sept 26	Oct 01	Oct 03	Oct 10	Oct 15	Oct 17	Oct 24	Oct 31
Day after 1st application	57	64	71	77	85	90	92	99	104	106	113	118	120	127	134

Table 2 Active ingredient and corresponding formulation product used for pesticide application.

Active Ingredient	**Formulation Product**
Chlorpyrifos	Lorsban 4-E (44.9% a.i.)
Permethrin	Pounce (25% a.i.)
Lambda-Cyhalothrin	Warrior (11.4% a.i.)

Table 3 Tank numbers and the pesticide concentrations for each application. LC = lethal concentration a certain percentage of the population is killed (determined in previous laboratory tests using *H. azteca* and *C. dilutus*), ". = *H. azteca*, *. = *C. dilutus*

Tank number	**Abbreviation**	**Application 1**	**Application 2+3**	**Application 4+5**
1 – 4	Control	0	0	0
5 - 8	T1	Environmentally relevant concentrations (Table 4)		
9 - 12	T2	LC10"	LC25"	LC50"
13 - 16	T3	LC10*	LC25*	LC50*

Table 4 Lethal concentrations pesticide concentrations were based on for the applications. Lethal concentration (LC) values were determined in 10-day toxicity tests using *H. azteca* and *C. dilutus.* Environmentally relevant concentrations were averaged from data listed on CEDEN database for the years 2011 and 2012 from monitoring studies, excluding runoff events (database accessed on 061213)

Pesticide	Pesticide concentration (ng/L)						
	H. azteca			*C. dilutus*			*Environmentally relevant concentrations*
	LC10	**LC25**	**LC50**	**LC10**	**LC25**	**LC50**	
Chlorpyrifos	58.10	66.95	77.15	128.52	192.07	267.11	7.53
Permethrin	48.56	55.01	62.30	161.78	284.41	533.97	5.76
Lambda-Cyhalothrin	0.14	0.17	0.21	37.78	43.31	49.65	3.50

Table 5 List of macroinvertebrate taxa identified in this study.

Class	**Phylum**	**Order**	**Family**	**Taxon**
Branchiopoda	Annelida	Oligochaeta	Naididae	Naididae ssp.
	Arthropoda	Diplostraca	Cyzicidae	*Cyzicus californicus*
Clitellata	Annelida	Hirundinea	Glossiphoniidae	Glossiphoniidae spec.
Crustaceae	Arthropoda	Amphipoda	Dogielinotidae	*Hyalella azteca*
		Trombidiformes	Hydrachnidae	Hydrachnidae spp.
Gastropoda	Mollusca	Pulmonata	Lymnaeidae	Radix spec.
			Planorbidae	Planorbidae ssp.
Insecta	Arthropoda	Coleoptera	Dytiscidae	Dytiscidae ssp.
				Hyderodes sp.
				Rhantus sp.
			Elmidae	Elmidae ssp.
			Hydrophilidae	Hydrophilidae ssp.
		Diptera	Chaoboridae	Chaoborus ssp.
			Chironomidae	Chironomidae spec
				Tanypodinae ssp.
			Culicidae	Anopheles spec.
				Culex spec.
			Simuliidae	Simuliidae ssp.
		Odonata	Anisoptera	Anisoptera ssp.
			Zygoptera	Zygoptera ssp.
		Rhynchota	Corixidae	Corixidae ssp.
			Notonectidae	Notonecta spec.
Turbellaria	Plathelminthes	Turbellaria	Turbellaria	Turbellaria ssp.

Table 6 List of zooplankton taxa identified in this study.

Class	Order/Subclass	Family	Taxon
Crustaceae	Cladocera	Bosminidae	Bosmina ssp.
		Chydoridae	*Chydorus sphaericus*
		Daphniidae	*Daphnia magna*
	Copepoda	Cyclopoida	Cyclopidae ssp.
		Calanoida	Calanoidae spec.
	Ostracoda		Ostracoda spec.
Rotatoria	Ploimida	Brachionidae	*Anuraeopsis fissa*
			Brachionus angularis
			Brachionus calycifloris
			Brachionus spec.
			Keratella cochlearis
			Keratella hiemalis
			Notholca spec.
			Platyias patulus
		Euchlanidae	Euchlanis spec.
		Gastropodidae	Ascomorpha spec.
		Mytilidae	*Mytilina mucronata*
		Trichocercidae	Trichocerca spec.

Figures

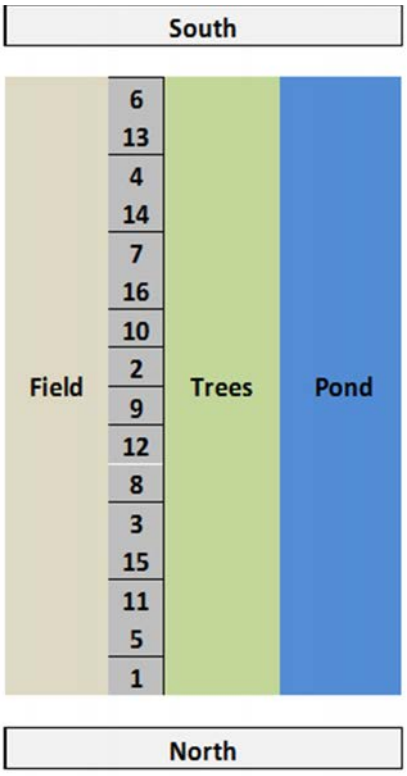

Figure 1Arrangement of tanks at the study site

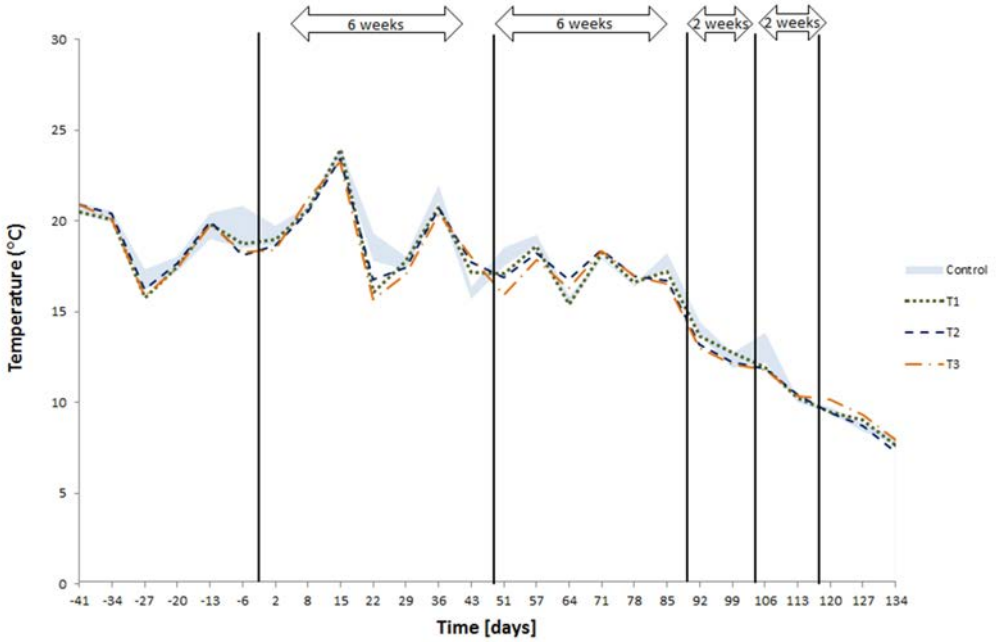

Figure 2 Water temperature over the course of the study period. Vertical lines indicate treatment dates. The three treatment levels are shown in comparison to the control area (represented by minimum and maximum values for each sampling day).

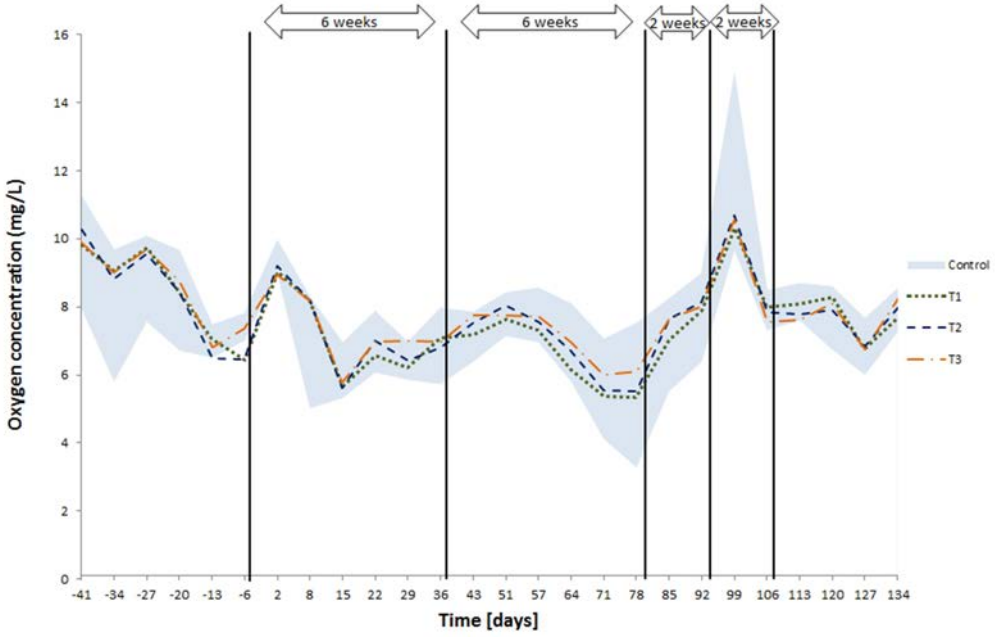

Figure 3 Oxygen concentration (mg/L) over the course of the study period. Vertical lines indicate treatment dates. The three treatment levels are shown in comparison to the control area (represented by minimum and maximum values for each sampling day).

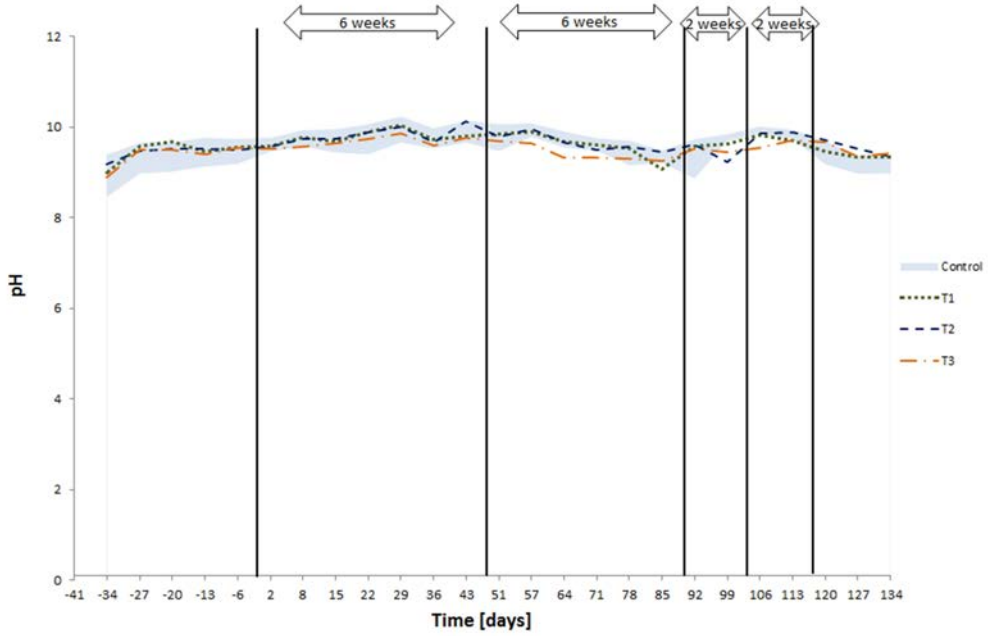

Figure 4 pH over the course of the study period. Vertical lines indicate treatment dates. The three treatment levels are shown in comparison to the control area (represented by minimum and maximum values for each sampling day).

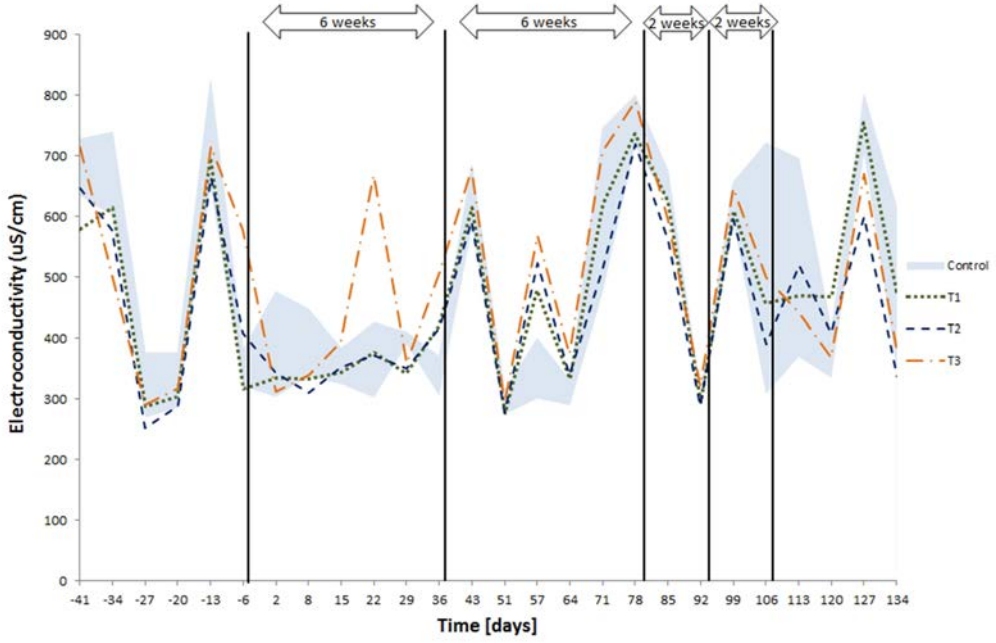

Figure 5 Electroconductivity (µS/cm) over the course of the study period. Vertical lines indicate treatment dates. The three treatment levels are shown in comparison to the control area (represented by minimum and maximum values for each sampling day).

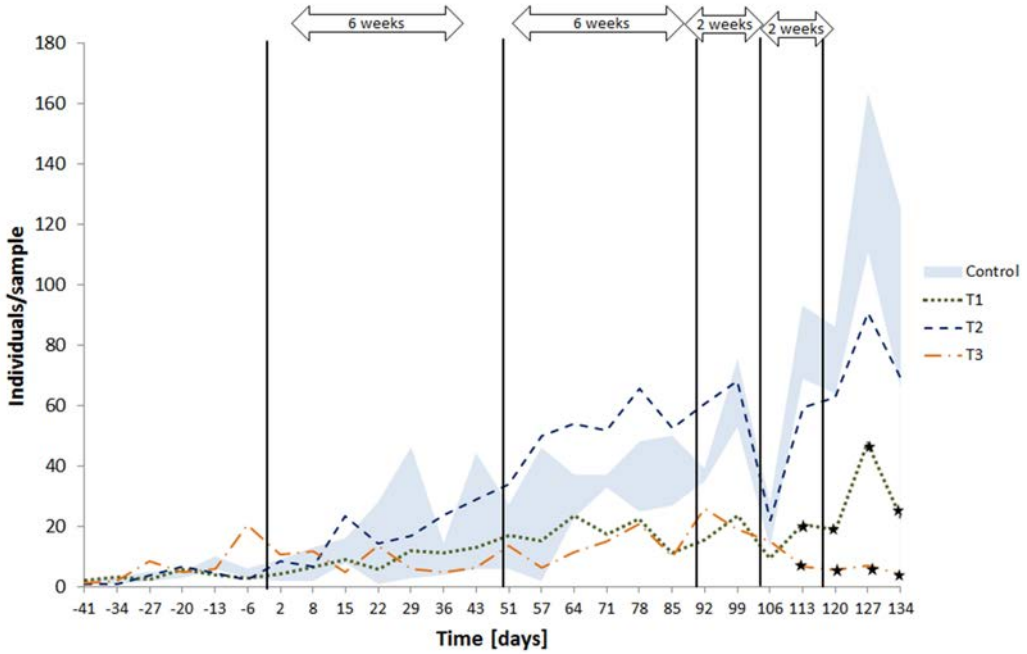

Figure 6 Average abundance of *Hyalella azteca* during the sampling period. Vertical lines indicate treatment dates. The three treatment levels are shown in comparison to the control area. Asterisks = significant deviation from the control (Dunnett's test; $p < 0.05$).

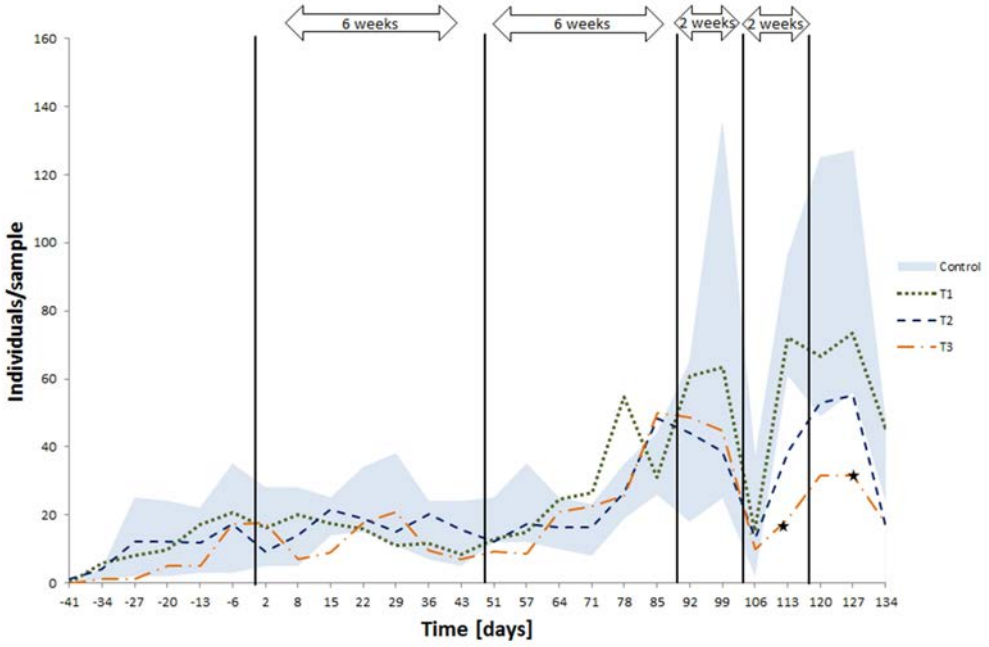

Figure 7 Average abundance of Zygoptera during the sampling period. Vertical lines indicate treatment dates. The three treatment levels are shown in comparison to the control area. Asterisks = significant deviation from the control (Dunnett's test; $p < 0.05$).

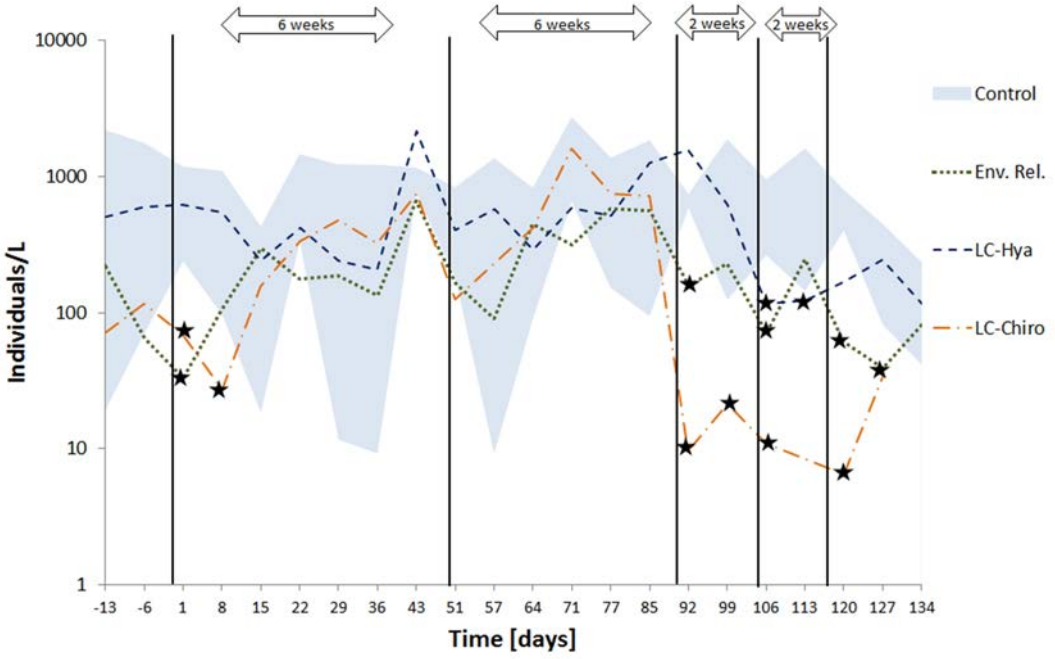

Figure 8 Average abundance of *Daphnia magna* during the sampling period. Vertical lines indicate treatment dates. The three treatment levels are shown in comparison to the control area. Asterisks = significant deviation from the control (Dunnett's test; $p < 0.05$).

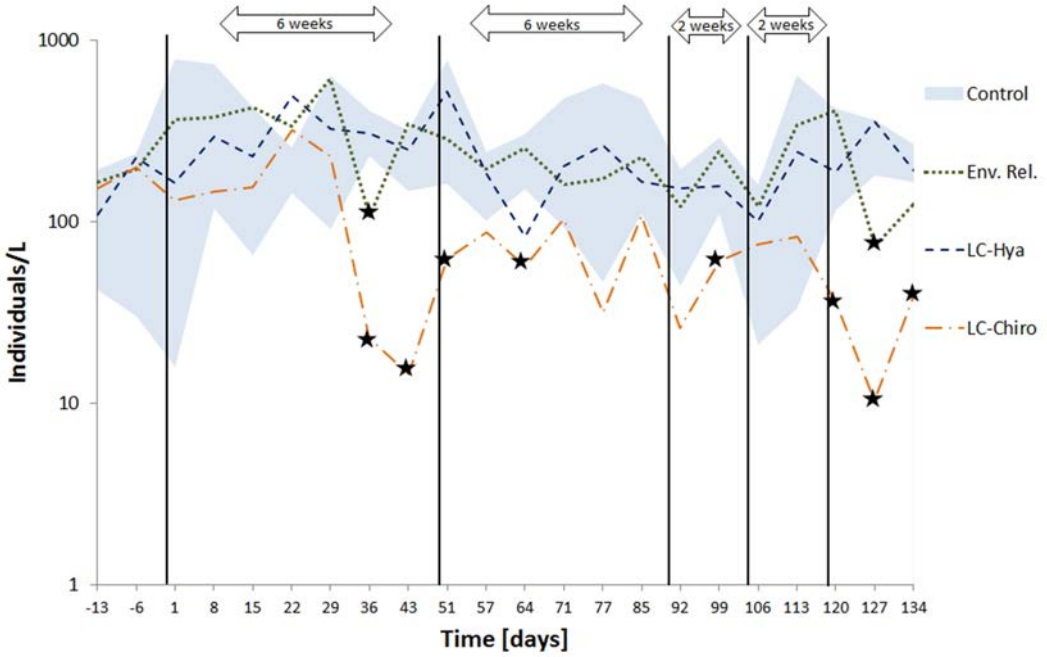

Figure 9 Average abundance of copepods during the sampling period. Vertical lines indicate treatment dates. The three treatment levels are shown in comparison to the control area. Asterisks = significant deviation from the control (Dunnett's test; $p < 0.05$).

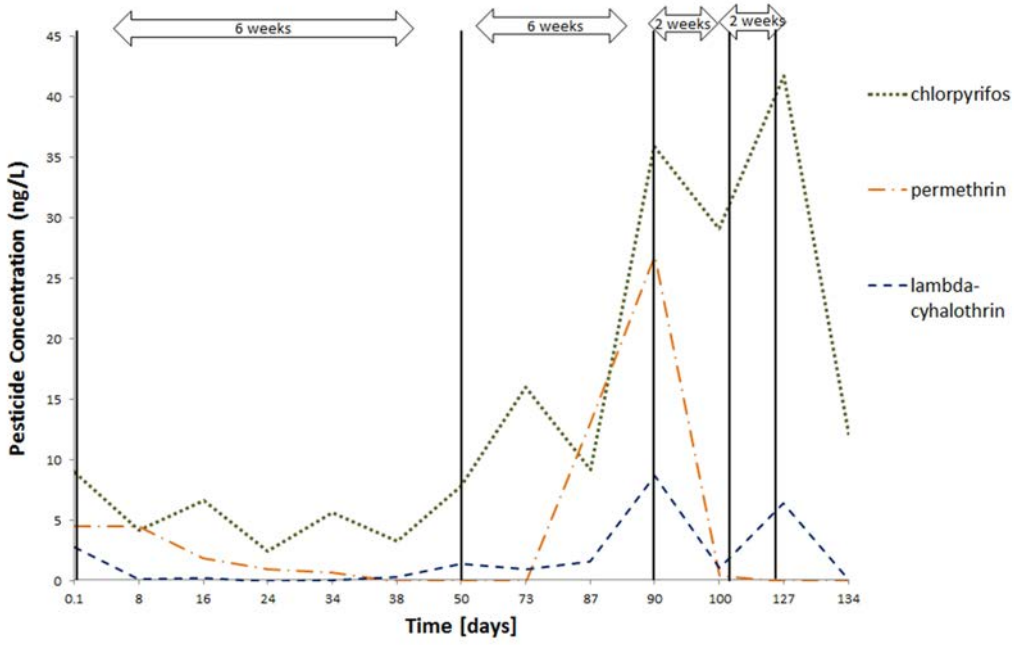

Figure 10 Average concentration (ng/L) of chlorpyrifos, permethrin, and lambda-cyhalothrin for treatment T1 in the water column on each sampling day. Vertical lines indicate treatment dates.

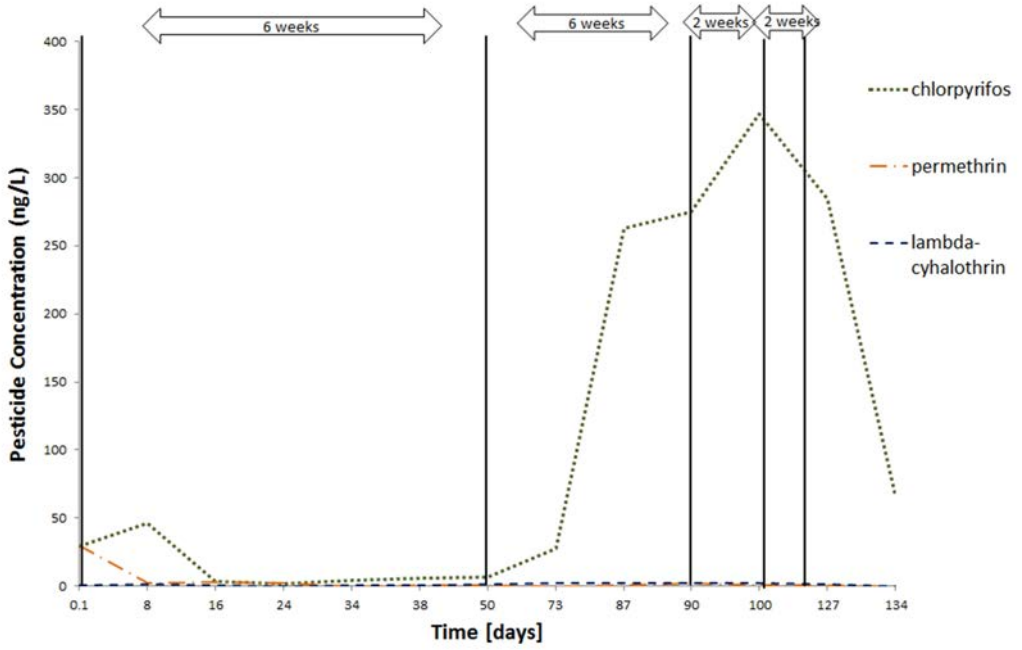

Figure 11 Average concentration (ng/L) of chlorpyrifos, permethrin, and lambda-cyhalothrin for treatment T2 in the water column on each sampling day. Vertical lines indicate treatment dates.

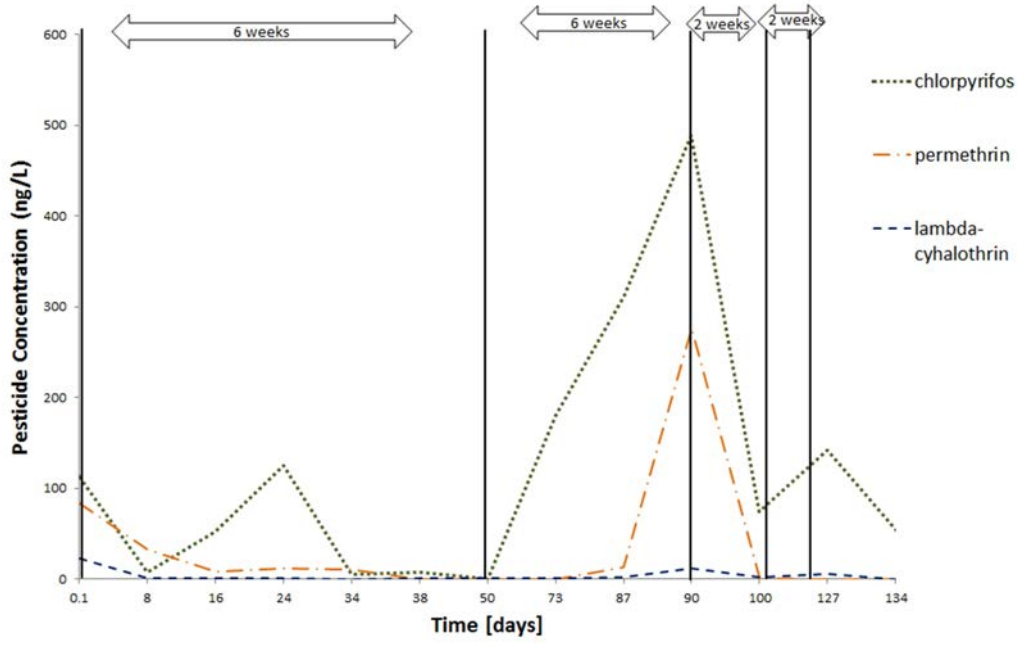

Figure 12 Average concentration (ng/L) of chlorpyrifos, permethrin, and lambda-cyhalothrin for treatment T3 in the water column on each sampling day. Vertical lines indicate treatment dates.

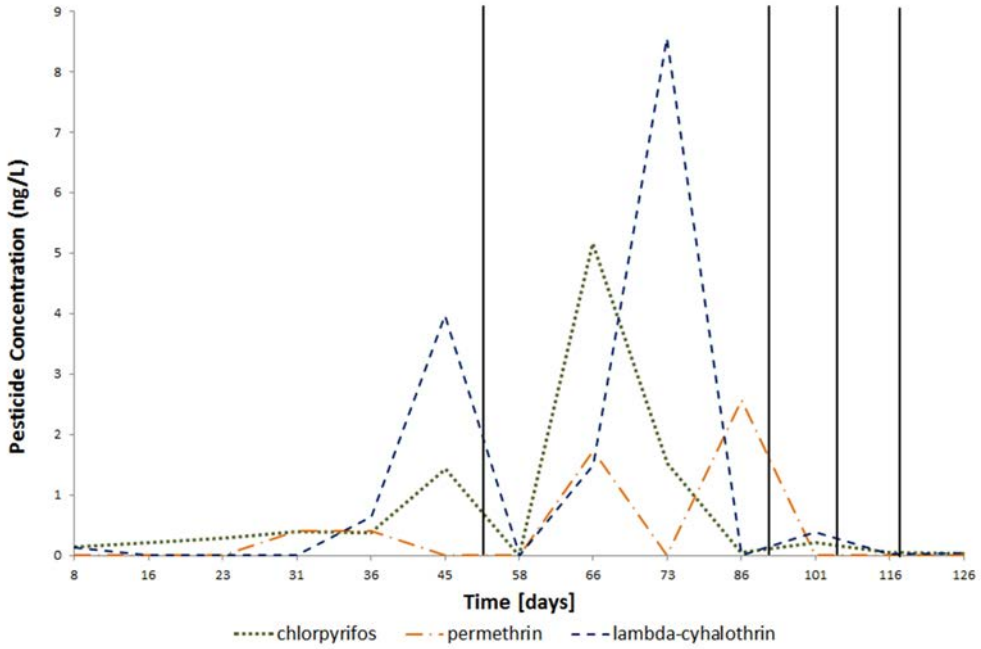

Figure 13 Average concentration (ng/L) of chlorpyrifos, permethrin, and lambda-cyhalothrin for treatment T1 in the sediment on each sampling day. Vertical lines indicate treatment dates. Please note, opposed to water column samples, sediment sampling started on day 8 and was conducted on weekly intervals in the first 10 sampling weeks, followed by sampling every other week from then on.

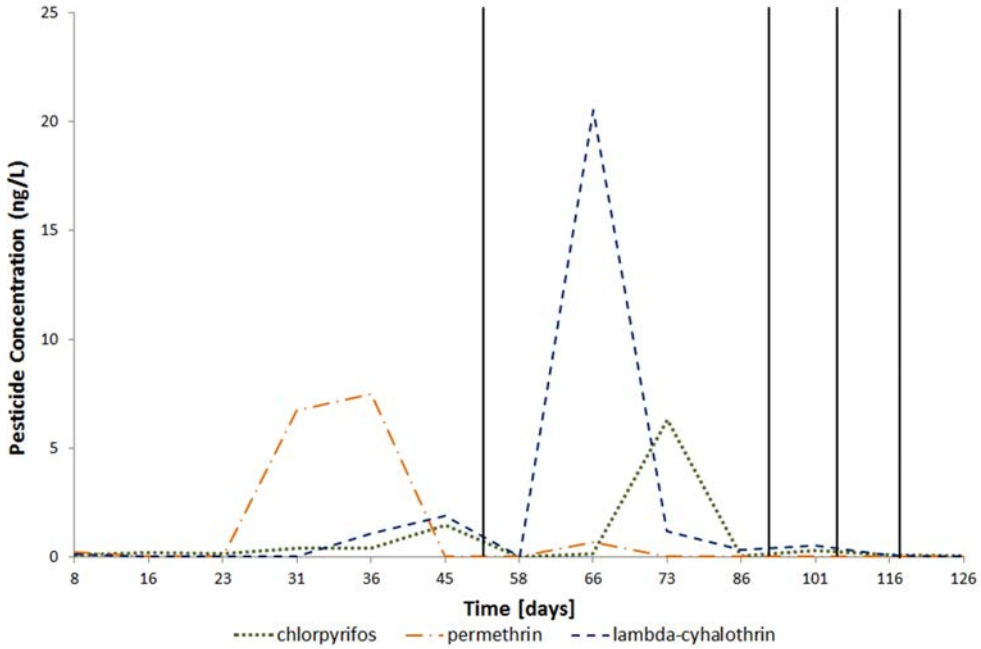

Figure 14 Average concentration (ng/L) of chlorpyrifos, permethrin, and lambda-cyhalothrin for treatment T2 in the sediment on each sampling day. Vertical lines indicate treatment dates. Please note, opposed to water column samples, sediment sampling started on day 8 and was conducted on weekly intervals in the first 10 sampling weeks, followed by sampling every other week from then on.

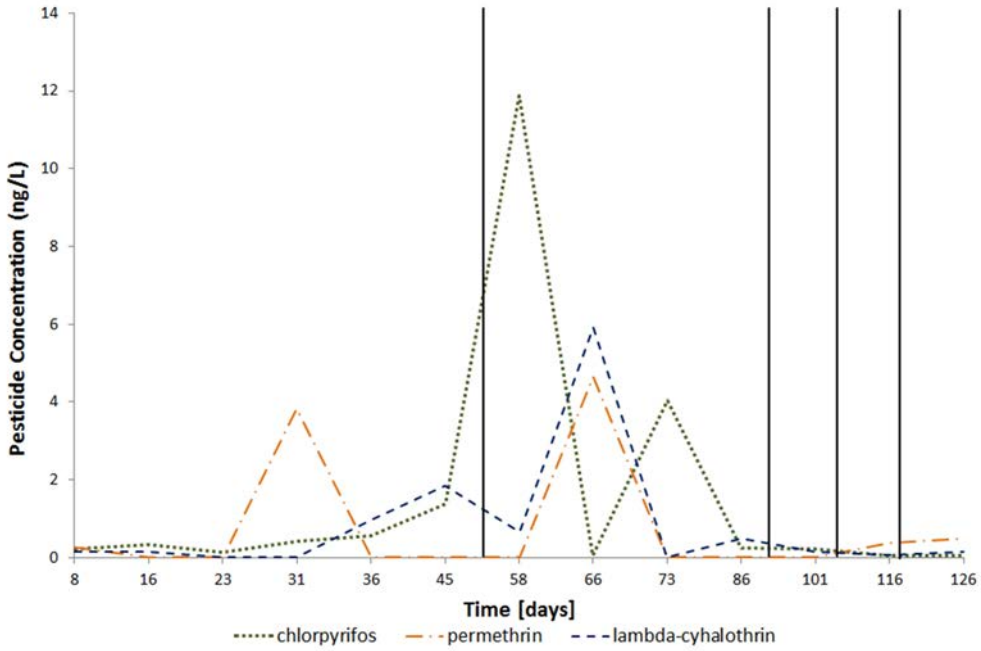

Figure 15 Average concentration (ng/L) of chlorpyrifos, permethrin, and lambda-cyhalothrin for treatment T3 in the sediment on each sampling day. Vertical lines indicate treatment dates. Please note, opposed to water column samples, sediment sampling started on day 8 and was conducted on weekly intervals in the first 10 sampling weeks, followed by sampling every other week from then on.

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