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Ecological Effects of Spring and Late Summer Applications of Lambda-Cyhalothrin on Freshwater Microcosms

R. P. A. Van Wijngaarden,[1] T. C. M. Brock,[1] P. J. van den Brink,[1,2] R. Gylstra,[2] S. J. Maund[3]

[1] Alterra, Wageningen University and Research Centre, P.O.Box 47, 6700 AA Wageningen, The Netherlands
[2] Department of Aquatic Ecology and Water Quality Management, Wageningen University, Ritzema Bosweg 32a, 6703 AZ, Wageningen, The Netherlands
[3] Syngenta Crop Protection AG, 4002 Basel, Switzerland

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Abstract. The aim of the study was to compare the effects of the pyrethroid insecticide lambda-cyhalothrin (treated at 10, 25, 50, 100, 250 ng active ingredient a.i./L) on a drainage ditch ecosystem in spring and late summer. Microcosms (water volume approximately 430 L) were established using enclosures in a 50-cm–deep experimental ditch system containing communities typical of macrophyte-dominated freshwater ecosystems. Effects on macroinvertebrates, zooplankton, phytoplankton, macrophytes, and community metabolism were assessed and evaluated using univariate and multivariate statistical techniques. The macroinvertebrate community responded most clearly to treatment and, as anticipated, insects and crustaceans were among the most sensitive organisms. Statistical analysis showed that the underlying community structure was significantly different between the spring and summer experiments. However, the most sensitive species (*Chaoborus obscuripes* and *Gammarus pulex*) were abundant in spring as well as in late summer. In spring and late summer, only slight and transient effects were observed at the community level in the 10-ng/L treatment. Overall, the study did not show substantial differences in the responses of sensitive taxa between spring and late summer treatments, and effects thresholds were similar irrespective of season of treatment.

Experiments in microcosms and mesocosms can be performed in higher-tier aquatic risk assessment for pesticides in the European Union when potential concerns are identified in the preliminary risk assessment (SANCO 2002). One recurring point of discussion regarding the use of microcosm and mesocosm data in higher-tier risk assessment has been the extent to which data from a single experiment can be extrapolated to potential for effects in the real world where multiple stressors may occur, environmental conditions may vary, biocoenoses may differ, and so on (Giddings *et al.* 2002).

One of the variables in the real world is that spraying of a pesticide may take place during different periods of the season. This may affect the impact of a toxicant on freshwater communities for several reasons. First, environmental conditions such as temperature, light, nutrients, biomass, and vegetation structure are seasonal. Changes in these conditions may influence the fate and bioavailability of compounds. Brock *et al.* (1992), for example, showed for chlorpyrifos that the mixing of the compound through the water column was strongly influenced by the vegetation structure in microcosms. For lambda-cyhalothrin, it has been demonstrated that different densities of macrophytes can have considerable influence on the fate and bioavailability of this compound (Hand *et al.* 2001; Leistra *et al.* 2003). Experiments with chlorpyrifos simulating different environmental conditions indicated that at higher water temperatures, and in combination with higher light intensities, dissipation rates increased (Van Wijngaarden *et al.* 2005). Second, species assemblages and the developmental stages of populations change with time. Several laboratory studies have suggested that this may affect the impact of a toxicant on freshwater communities (Kindig *et al.* 1983; Swartzman *et al.* 1990; Taub *et al.* 1991). One explanation for this is that, in general, juvenile growth stages are more sensitive to toxicants than older ones (Mayer and Ellersieck 1986; Hutchinson *et al.* 1998). Laboratory studies also indicate that temperature and toxicity are positively correlated for most chemicals: toxicity increases as temperature increases (Mayer and Ellersieck 1986).

In temperate climates in springtime, surface water ecosystems are typically in a developmental stage (*i.e.*, young, sensitive organisms; little plant biomass leading potentially to higher bioavailability of the toxicant) and exposed to cool environmental conditions (*i.e.*, less potential for toxicity, lower degradation). In late summer, these systems have matured (*i.e.*, older, less sensitive organisms; more plant biomass leading to potentially lower bioavailability of toxicant) and are exposed to warmer conditions (*i.e.*, more potential for toxicity and higher degradation). Hence, counteracting factors may

Correspondence to: R. P. A. van Wijngaarden; *email:* rene.vanwijngaarden@wur.nl

simultaneously influence the outcome of a pesticide exposure, and this may make it difficult to predict the relative sensitivity of systems tested under spring conditions compared with those tested later in the year.

The contribution of seasonal factors for determining risks is an important question in ecologic risk assessments of pesticides (Campbell *et al.* 1999; Giddings *et al.* 2002). Nevertheless, relatively few microcosm studies with pesticides have investigated the influence of season of application on the response of aquatic ecosystems. In outdoor microcosm studies that focused on the effects of 10 μg/L of the herbicide atrazine on plankton communities during different periods of the year, it appeared that the clear water phase (June) was the period when the algal communities were most sensitive to restructuring by atrazine, whereas they were the least sensitive during the spring blooms (Bérard *et al.* 1999). In experimental ponds, carbaryl treatment at different stages in the seasonal cycle induced distinct recovery patterns in zooplankton communities. It was suggested that temperature, competitive interactions, and population trends were significant factors influencing recovery of the zooplankton (Hanazato and Yasuno, 1990). Both previous studies worked at concentration levels that were well above the threshold concentration for direct effects and indicated differences in community responses related to season. However, a study with pentachlorophenol in lake enclosures, which also included concentrations around the threshold level, indicated that direct effects on planktonic communities varied little with season. It was suggested that season was not a particularly important factor for ecologic risk assessment and the determination of ecological threshold levels (Willis *et al.* 2004).

The aim of the present study was to compare the effects of a spring insecticide treatment with the effects of a late summer treatment with the same compound. We focused on the questions of (1) whether sensitivities of populations and communities were different, specifically at concentration levels near those used as thresholds in regulatory risk assessments and (2) whether above these concentrations, direct and indirect effects and recovery times were different.

Microcosms were established using enclosures in a 50-cm–deep experimental ditch system containing natural communities typical of macrophyte-dominated drainage ditches. The pyrethroid insecticide lambda-cyhalothrin was used as the test compound. Lambda-cyhalothrin was chosen because it is highly lipophilic and tends to bind rapidly and strongly to organic materials (Maund *et al.* 1998; Leistra *et al.* 2003). Consequently, seasonal changes in biomass of plants and algae in aquatic ecosystems may affect exposure to this compound. Furthermore, lambda-cyhalothrin is highly toxic to some groups of aquatic organisms, particularly insects and crustaceans (Maund *et al.* 1998; Schroer *et al.* 2004). These taxonomic groups provide a range of univoltine and multivoltine species, giving an opportunity to test different life stages present in the spring and the late summer experiments.

The work described in this article is part of a series of concurrent experiments done in ditch enclosures to study the fate and effects of lambda-cyhalothrin under varying environmental conditions. Leistra *et al.* (2003) studied the fate of the compound. Separate enclosures were installed to follow the dynamics of lambda-cyhalothrin in detail. Roessink *et al.* (2005) reported the effects of lambda-cyhalothrin in enclosures of different trophic states. Schroer *et al.* (2004) investigated the toxicity of lambda-cyhalothrin to freshwater invertebrates using data from short-term laboratory toxicity tests and *in situ* bioassays and population effects observed in the enclosures.

Materials and Methods

Experimental Outline

The experiments were carried out in macrophyte-dominated ditches (length 40 m and width 2.80 m at water surface) at the Sinderhoeve experimental station, Renkum, The Netherlands. The spring experiment was performed in May through June and the late summer experiment in August through September of 2000. In the spring experiment, 1 ditch from the 12 available on the site was selected on the basis of having evenly distributed, well-developed macrophyte coverage. In the late summer experiment, the ditch selected was similar to that used in the spring experiment (based on vegetation structure and composition). Twelve enclosures (polycarbonate, translucent cylinders: diameter 1.05 m, height 0.9 m, water volume c. 0.43 m^3) were placed in an evenly distributed row down the center of the ditch. The cylinders were pushed approximately 15 cm into the sandy-loam sediment and contained a water column 0.5 m deep. In both experiments, the enclosures were placed in the ditches 3 weeks before treatment. In each season, 3 applications of lambda-cyhalothrin were made at weekly intervals. Treatment started on May 16 for the first experiment and on August 15 for the second. The formulated product KARATE (100 g lambda-cyhalothrin/L as capsule suspension, Syngent Basel, Switzerland) was applied at nominal concentrations of 10, 25, 50, 100, and 250 ng active ingredient (a.i./L) per liter, and each treatment was duplicated. Two enclosures served as controls and were only treated with water. Treatments were randomly assigned to the enclosures. Treatments were made by pouring a carefully measured volume of treatment solution into the enclosures, after which the water column was gently stirred to mix the compound throughout the water column without disturbing the sediment. Methods of application and chemical analysis are further described in Leistra *et al.* (2003).

Fate

Nominal initial treatment concentrations were based on measured concentrations of lambda-cyhalothrin in the treatment solutions and the water volume of the enclosures. Initial measured concentrations were assessed by taking depth-integrated water samples (with a Perspex [Lucite] tube: (United Kingdom) diameter 4 cm and length 50 cm) at 1 hour after treatment. Two water-column samples were taken and pooled from each enclosure.

Approximately 100 mL of the sampled water was stored in preweighed bottles and taken to the laboratory for analysis. In the laboratory, the bottles were weighed, and 30 mL distilled hexane was added. After weighing, the water and hexane were thoroughly mixed on a shaking apparatus for 15 minutes. The hexane layer was isolated in preweighed tubes, after which the tubes were weighed again. Hexane was evaporated under a flow of pressurized air. The residue was then dissolved in 1 mL distilled hexane. This was mixed on a vortex and transferred to a gas chromatography vial. Lambda-cyhalothrin was analyzed using a Hewlett Packard 5890 gas chromatograph (Ausadale, PA, USA) equipped with an electron-capture detector. For further details, see Leistra *et al.* (2003).

Macroinvertebrates

Artificial substrates were used to sample the macroinvertebrate community. These consisted of two litter bags (see section Decomposition), two multiplates, and two pebble baskets. A detailed description of the former two substrates is given in Brock *et al.* (1992). Substrates were collected from each enclosure at intervals of 2 or 3 weeks. At the time of sampling, the artificial substrates were gently retrieved from the enclosures using a net to prevent the escape of organisms. Pebble baskets and multiplates were first washed in a container to remove invertebrates. Subsequently, the macroinvertebrates retrieved with the net, from both substrates, and from the litter bags were carefully sorted by hand. Organisms that were alive were identified and counted, after which they were released again into their original enclosures. Data from the artificial substrates and litter bags were pooled for further analysis.

Phytoplankton and Zooplankton

Plankton were sampled at weekly intervals from each enclosure using a Perspex tube (length 0.4 m and volume 0.8 L). Several subsamples were collected from each enclosure until a 10-L sample had been obtained. Five liters were used for the zooplankton analysis. The 5-L sample was concentrated by means of a 55-μm mesh net (Hydrobios, Kiel, Germany) and was preserved with formalin (end volume c. 4%). Of the remaining 5 L, 1 L was collected for chlorophyll-a analysis to estimate phytoplankton biomass.

The total number of cladocerans, ostracods, and juvenile and adult copepods was counted under a binocular microscope at 25x magnification. Numbers of rotifers and copepod nauplii were determined by counting a known volume using an inverted microscope (100x to 400x magnification). Rotifers and cladocerans were identified to the lowest practical taxonomic level. Copepods were divided into calanoids and cyclopoids. Abundances were adjusted to numbers per liter.

Phytoplanktonic chlorophyll-*a* measurements were made by concentrating a 1-L water sample over a glass-fiber filter (Schleicher and Schuell GF_{52}, mesh size 1.2 μm; Dassel, Germany). Filters were stored in Petri dishes, wrapped in aluminium foil, and kept in a deep-freezer at a temperature <–20 °C until analysis. Extraction of the pigments was performed using a spectrophotometer (Beckman, DU-64; Beckman Coulter, Mijdrecht, The Netherlands) according to the method of Moed and Hallegraeff (1978).

Periphyton

Glass slides (7.6 × 2.6 cm) were used as artificial substrates for sampling the periphyton. The slides were vertically positioned in a frame at a fixed depth of approximately 25 cm below the water surface of each enclosure. The substrates were introduced 15 or 16 days before the first application. Substrates were collected on days –1, 6, 13, 20, 27, and 41.

At sampling, a maximum of 5 slides/enclosure were collected to measure the amount of chlorophyll-*a* as an estimate of periphytic algae biomass. The slides were brushed and washed with tap water to collect the periphyton. The chlorophyll-*a* content of the water–periphyton suspension was processed and analyzed as described previously for phytoplankton.

Macrophytes

At days –7 (spring experiment) and –11 (late summer experiment) the above-sediment macrophyte biomass of two representative plots (0.25 × 0.25 m) in the experimental ditches (but not inside the enclosures) was sampled. At this time, biomass in the area of the ditch outside the enclosures was similar to that inside the enclosures. At the end of both experiments, the complete above-sediment vegetation within the enclosures was harvested. Before drying (for 24 hours at 105°C), the plant material was rinsed under tap water to remove loosely attached materials such as sediment particles and macroinvertebrates.

Bioassays

The crustacean *Asellus aquaticus* and the insect *Chaoborus obscuripes* were tested in *in situ* cage experiments. We used these relatively sensitive species (Schroer *et al.* 2004) for comparison of the acute effects of lambda-cyhalothrin between the two experiments and to determine whether potential recovery was different between the two seasons. *A. aquaticus* and *C. obscuripes* were collected in the field and kept in aquaria in the laboratory for several weeks before use. One week before the experiments started, the organisms were acclimated to the experimental conditions by transferring them to containers located in one of the experimental ditches.

The bioassay cages used were constructed of stainless steel gauze (mesh size 0.5 mm, length 33 cm, diameter 6 cm, volume: 930 cm^3;). In each cage, 25 or 30 adult *A. aquaticus* (mean size [± SD] 5.9 [± 1.4] mm in spring and 5.7 [± 1.3] mm in late summer) were introduced. *Populus x canadensis* leaves (ca. 1 g dry weight) were supplied to these cages to provide sheltering substrates for *A. aquaticus*. Thirty specimens (fourth instar) were used per cage in the *C. obscuripes* bioassays, and two cages were introduced into each enclosure.

Two bioassays were performed, one directly after the first application (acute effects bioassay) and a second after the third application (recovery bioassay). *A. aquaticus* and *C. obscuripes* in the first bioassays were introduced at day –1. After application of the insecticide, the surviving organisms were counted and reintroduced in the bioassay on days 1, 2, 3, and 6. In the recovery bioassays, tests started 0, 4, and 8 days after the last application. Effects were scored after 4 or 5 days of exposure. After counting, the surviving organisms were not reintroduced, but fresh test organisms were used for each recovery bioassay.

To promote water exchange between enclosures and cages, cages were gently pulled up and down the water column at regular intervals. Because of the known rapid dissipation of lambda-cyhalothrin from the water, this was done most intensively on the day of application (after 1, 2, 4, and 8 hours) and thereafter on the days of data collection. The intention of this mixing was to ensure that the insecticide exposure patterns within the cages followed that of the enclosures as closely as possible.

Results of the bioassays were quantified by calculating percentile effect concentrations (EC*x* and LC*x* values for immobility and mortality, respectively). Bioassay results of individual replicates were used for the EC and LC calculations, and initial nominal concentrations were used as input for the regression model (Schroer *et al.* 2004).

Decomposition

Decomposition of particulate organic matter (POM) was studied using leaf litter bags made up with *Populus* leaves. Before use, the leaves had been soaked in water three times for 2 days to remove the more easily soluble humic compounds and then dried in an oven for 72 hours at 60°C.

A portion of the dried leaves, 2 g dry weight, was enclosed in each litter bag and consisted of a glass Petri dish (diameter 11.6 cm) closed with a cover of stainless steel wire (mesh size 0.7 mm), in which two

holes (diameter 0.5 cm) were made to allow invertebrates to enter. In each enclosure, two litter bags were placed on the sediment surface for a period of 2 weeks. At the end of each incubation period, the litter bags were gently retrieved from the enclosures and emptied in a white tray to separate POM from adhering sediment particles and macroinvertebrates by rinsing with tap water. The plant material was dried in aluminium foil at a temperature of 105°C. After 24 hours, dry weight was determined. Macroinvertebrates were counted and included in the macroinvertebrate sampling scores (see Macroinvertebrates section).

Community Metabolism

As an indicator of the overall oxygen metabolism of primary producers, dissolved oxygen (DO) was measured. Changes in primary productivity affect pH, alkalinity, and electrical conductivity (EC). Because DO, pH, alkalinity and EC are often found to be highly correlated, and because indirect treatment effects can be regarded as a stress syndrome (Giddings 1982), these end points were monitored on a weekly basis. Measurements were made at a depth of 10 cm in the approximate center of each enclosure. DO was measured with a WTW Oxi330 portable oxygen meter (Retch, Ochten, The Netherlands). Electrical conductivity was measured with a WTW LF191 conductivity meter (Retch). pH was measured with a WTW PH197 portable pH-meter (Retch). The alkalinity of 100-mL water samples taken at a depth of 10 cm was measured by titration with 0.02 N HCl to pH 4.2.

Data Analysis

Before analysis, the macroinvertebrate and the zooplankton data sets were, respectively, $\ln(2x+1)$ and $\ln(10x+1)$ transformed, where x was the abundance value. This was done to down-weight high abundance values and approximate a normal distribution of the data (Van den Brink *et al.* 2000).

No observed effect concentration (NOEC) calculations at parameter or taxon level were derived using the Williams test (analysis of variance $p < 0.05$; Williams 1972). Analyses were made with the Community Analysis computer program (Hommen *et al.* 1994). ECx calculation methods on the results of the bioassays have been described by Schroer *et al.* (2004).

Effects of the lambda-cyhalothrin treatments on the community level of zooplankton and macroinvertebrates were analyzed by the principal response curves (PRC) method, which is based on the redundancy analysis ordination technique, the constrained form of principal component analysis (Van den Brink and Ter Braak 1998, 1999). For a complete description and discussion of the PRC method, the reader is referred to Van den Brink and Ter Braak (1998, 1999). The PRC analysis was performed using the CANOCO for Windows software package, version 4 (Ter Braak and Smilauer 1998). In the CANOCO computer program, redundancy analysis is accompanied by Monte Carlo permutation tests to assess the statistical significance of effects of the explanatory variables on species composition of the samples (Van den Brink *et al.* 1996). The significance of the PRC diagram, in terms of displayed treatment variance, was tested by Monte Carlo permutation of the entire time series in the redundancy analysis from which the PRC is obtained using an F-type test statistic based on the eigenvalue of the component (Van den Brink and Ter Braak 1999).

Monte Carlo permutation tests were also performed by sampling date using the ln-transformed treatment levels as the explanatory variable (Van den Brink *et al.* 1996). This allowed the significance of the treatment regime to be tested for each sampling date. We also determined which treatments differed significantly from the controls so as to infer the NOEC at the community level ($NOEC_{community}$). The $NOEC_{community}$ calculations were done by applying the Williams test to the sample scores of the first principal component of the principal component analysis of each sampling date in turn (the rationale for this can be found in Van den Brink *et al.* 1996).

Monte Carlo permutation tests were also performed for each sampling date to test whether the communities differed significantly between seasons and whether there was interaction between the factors "treatment" and "season." The model used was

$$y_{d(j)ka} = y_{0k0} + T_d + S_a + (T_d * S_a) + \varepsilon_{d(j)ka},$$

where $y_{d(j)ka}$ is the abundance of species k in treatment d of replicate j of season a and y_{0k0} is the abundance of species k in the reference treatment (control = 0) and reference season (spring = 0). T_d indicates the effect of the treatment and S_a the effect of the season. The $T_d{*}S_a$ factor denotes the effect of the interaction term of treatment and season. $\epsilon_{d(j)ka}$ is an unknown error term associated with observation $y_{d(j)ka}$. Within CANOCO for each sampling date separately, we tested "treatment" by introducing ln-transformed (see Van den Brink *et al.* 1996 for details) treatment levels as explanatory variable and nominal variables denoting "season" plus its "interaction with treatment" as covariables. We tested "season" for each sampling date by introducing a nominal variable denoting "season" as explanatory variable and the treatment variable and its "interaction with season" as covariables. We tested "interaction" by entering the "interaction between season and treatment" as explanatory variables and the ln-transformed treatment levels and the nominal variable denoting "season" individually as covariables.

Results

General Description of Test Systems

The general characteristics of the test systems are listed in Table 1. Water temperatures were almost similar in spring and late summer. Temperatures tended to increase during the spring experiment, whereas they tended to decrease in the late summer experiment.

The macrophyte stands in the ditches selected were dominated by *Myriophyllum spicatum*, with *Elodea nuttallii* codominant in some patches; *Sagittaria sagattifolia* was also common. The latter two species became more dominant in late summer. Mean macrophyte biomass at the beginning of the experiments was almost 2.5 times higher in late summer than in spring (Table 1). The organic matter content of the upper sediment layer was more or less similar for both experiments (Table 1). Dissolved organic carbon in the water column was somewhat higher in spring (Table 1).

The ditches are representative of mesotrophic freshwater bodies. As an indication of nutrient levels in the systems, geometric mean values in the enclosures measured during the time span of the spring experiment were 0.02 mg/L (NH_4^+) and 0.03 mg/L ($NO_3^- + NO_2^-$); for ortho-P, concentrations were 0.04 mg/L (Roessink *et al.*, 2005). Overall, community metabolism was higher in spring because dissolved oxygen levels and pH were relatively high and electrical conductivity and alkalinity levels were relatively low compared with those in late summer (Table 1).

Table 1. Characterization of the enclosures set up in spring and late summer[a]

Characteristics	Spring	Summer
Sediment[b]		
Organic matter (%) 0–2 cm upper layer	26	23
Water		
DOC (mg C/L)	9.1	7.9
DO (mg/L)	9.7 ± 0.9	5.8 ± 0.5
pH	9.9 ± 0.1	7.4 ± 0.2
EC (μS/cm)	124 ± 8	174 ± 9
Alkalinity (meq/L)	1.06 ± 0.10	1.50 ± 0.10
Temperature (°C)	17.5 ± 1.6	17.9 ± 1.1
Macrophytes		
biomass (g/m^2 dw)	104	241

[a] Values are from the control enclosures. Mean values (± 95% CI) during the time span of the experiments are given for water quality end points. Mean macrophyte biomass represents values of the vegetation in the ditches housing the enclosures at start of the experiments.
[b] Values at start of experiments from Leistra *et al.* (2003).
CI = Confidence Interval.
DO = Dissolved oxygen.
DOC = Dissolved organic carbon.
dW = Dry weight.
EC = Electrical conductivity.

Table 2. Measured concentrations of lambda-cyhalothrin (ng a.i./L) in water samples collected 1 hour after application[a]

	Spring						Summer					
	Treatment						Treatment					
	1		2		3		1		2		3	
Intended	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
10	–	–	7	17	12	15	10	19	9	61	11	11
25	–	–	32	48	25	28	26	28	25	31	25	26
50	–	–	25	35	–	21	52	52	45	50	44	48
100	–	–	82	88	51	90	91	99	76	82	84	91
250	–	–	161	185	129	176	241	–	214	228	210	222

[a] Data for each replicate (R1 and R2) are given.
– = Sample lost.

Fate

Despite efforts to promote mixing after application, measured concentrations in water column samples taken 1 hour after application indicated very high spatial variability (Table 2). The mean overall treatments ± SD was 109% ± 81% of the intended concentrations. It was therefore considered more appropriate to use the nominal initial concentrations (concentrations in enclosures as calculated from measured concentrations in dose solutions and water volumes in enclosures) as estimators of the initial exposure concentrations. Overall, nominal initial concentrations were near to the intended nominal concentrations (Table 3).

The lowest treatment level (10 ng a.i./L) is of special importance for the response of the very sensitive insect species *C. obscuripes* (48-hour median effective concentration [EC_{50}] 2.8 ng a.i./L [Schroer *et al.* 2004]). Variations at this treatment level in actual exposure concentrations might strongly influence the response of this species. Measurements showed that in two of the three applications, the mean initial concentrations of the 10 ng/L-treatment level were higher in the late summer experiment than in the spring experiment. On average, the three summer applications were 13% higher than in spring.

Leistra *et al.* (2003) showed that lambda-cyhalothrin dissipated rapidly from the water column. In spring, lambda-cyhalothrin concentrations in the water were 24% (range 23% to 25%) of the initial nominal concentration by 1 day after treatment. After 7 days, only 2% (range 1.8% to 2.1%) of the initial nominal concentration could be detected in the water column.

In late summer, concentrations of lambda-cyhalothrin in the water were decreased to 34% (range 31% to 37%) of the initial nominal concentrations after 1 day and had further decreased to 0.6% (range 0.4% to 0.7%) after 7 days (Leistra *et al.* 2003). Rates of dissipation of lambda-cyhalothrin from the water column were considered to be comparable irrespective of season (Leistra *et al.* 2003).

Macroinvertebrates

Community Composition in Spring and Late Summer. A total of 67 macroinvertebrate taxa were identified in the enclosures in the spring experiment. In late summer, this was

Table 3. Intended and nominal initial concentrations of lambda-cyhalothrin (ng a.i./L)[a]

	Nominal			
	Spring		Late summer	
Intended	Replicate 1	Replicate 2	Replicate 1	Replicate 2
10	10 (9–11)	9 (6–10)	10 (9–11)	11 (8–13)
25	26 (23–29)	25 (24–26)	24 (23–25)	24 (21–30)
50	53 (45–59)	50 (43–56)	48 (42–58)	50 (39–59)
100	107 (91–134)	112 (106–118)	103 (97–107)	96 (86–107)
250	267 (253–293)	277 (252–309)	261 (240–279)	277 (266–284)

[a] Nominal initial concentrations are the average (range) of the three applications per experiment. Nominal initial concentrations were based on measured concentrations of lambda-cyhalothrin in dose solutions and water volumes of enclosures.

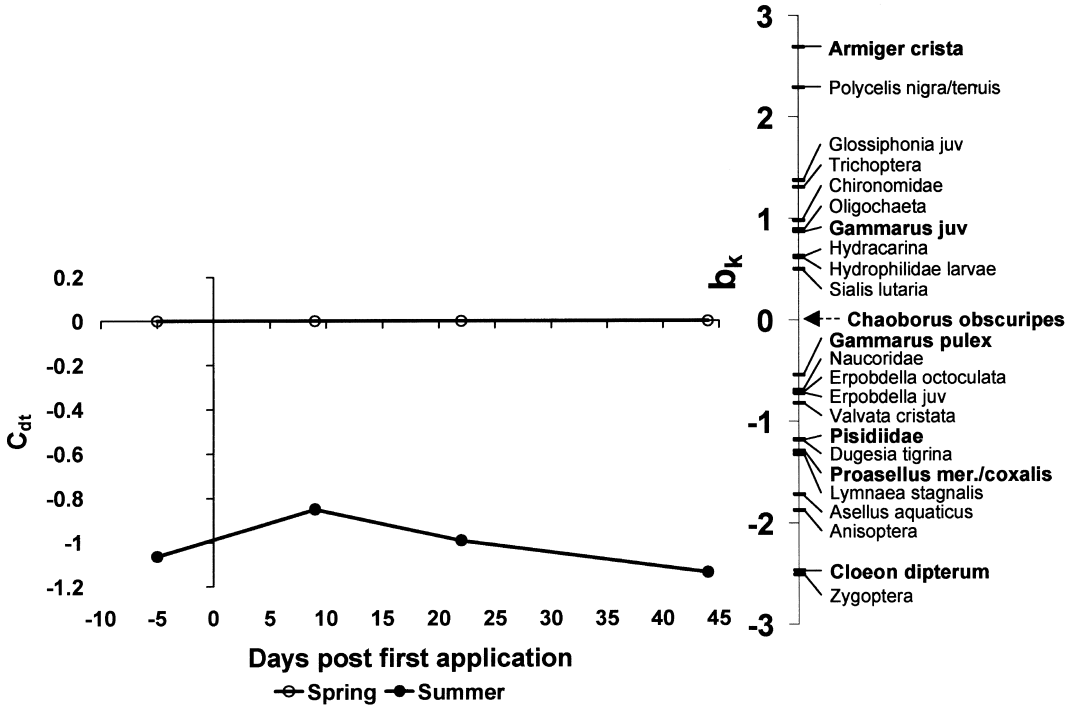

Fig. 1. PRCs indicating differences in macroinvertebrate species composition in spring and late summer in macrophyte-dominated control enclosures. Of all variance, 19% could be attributed to sampling date; this is displayed on the horizontal axis. Forty-eight percent of all variance could be attributed differences between seasons. Of this variance, 61% is displayed on the vertical axis of the PRC diagram. Abundant species are indicated in bold. Abundant species were considered as 25% of all species having the highest abundance numbers per sampling date and were present at that level for three of the four samplings in at least one of both seasons. The vertical axis represents the differences in community structure between treatment and controls expressed as regression coefficients (C_{dt}) of the PRC model. Species with a weight (b_k) between 0.5 and –0.5 are not shown (except for *C. obscuripes*). PRC = principal response curve.

slightly less with 61 taxa identified. Statistical testing indicated that species composition as a whole in late summer differed significantly from that of spring (Monte Carlo permutation test $p < 0.05$). In late summer, the snail *Armiger crista*, the flatworm *Polycelis nigra/tenuis*, juvenile *Glossiphonia* (leech), and trichopterans (caddis flies) were present in relatively low numbers. In contrast, the molluscs Pisidiidae and *Lymnaea stagnalis*, the flatworm *Dugesia tigrina*, the crustaceans *A. aquaticus* and *Proasellus meridianus/coxalis*, and the insects *Cloeon dipterum*, Anisoptera, and Zygoptera were present in relatively higher numbers compared with the spring experiment (Fig. 1). The species in the cluster *Chironomidae–Valvata cristata* (Fig. 1) contained, in addition to those species that were present in both seasons, also the species that had very low abundance values throughout the year. The potentially most sensitive species, *i.e.*, *C. obscuripes, Gammarus* juveniles, and *G. pulex*, were relatively abundant in both experiments (species weight (b_k) between –1 and 1; Fig. 1).

Community Level Response. The multivariate analyses indicated that the macroinvertebrates showed significant treatment-related effects compared with the controls in spring as well as in late summer (Table 4). In both experiments, impacts of treatments occurred directly after the first treatment

Table 4. Monte Carlo permutation tests on PRC coordinates (p values) for the spring and late summer macroinvertebrate and zooplankton communities[a] and $NOEC_{community}$ calculations (William tests $p < 0.05$)

	Macroinvertebrates				Zooplankton			
	Spring		Summer		Spring		Summer	
Week	p value	$NOEC_{community}$	p value	$NOEC_{community}$	p value	$NOEC_{community}$	p value	$NOEC_{community}$
−1	>0.05	250	>0.05	250	>0.05	≥250	>0.05	≥250
1	<0.001	<10	<0.001	10	>0.05	≥250	>0.05	≥250
2	–	–	–	–	>0.05	≥250	0.025	≥250
3	<0.001	50	0.006	50	≤0.005	25	0.010	≥250
4	–	–	–	–	>0.05	≥250	0.050	≥250
5	–	–	–	–	–	–	>0.05	≥250
6	0.016	100	<0.001	50	>0.05	≥250	–	–

[a] Indicating significance of the treatment regime of lambda-cyhalothrin significance of the different treatment levels. NOECs in ng/L.

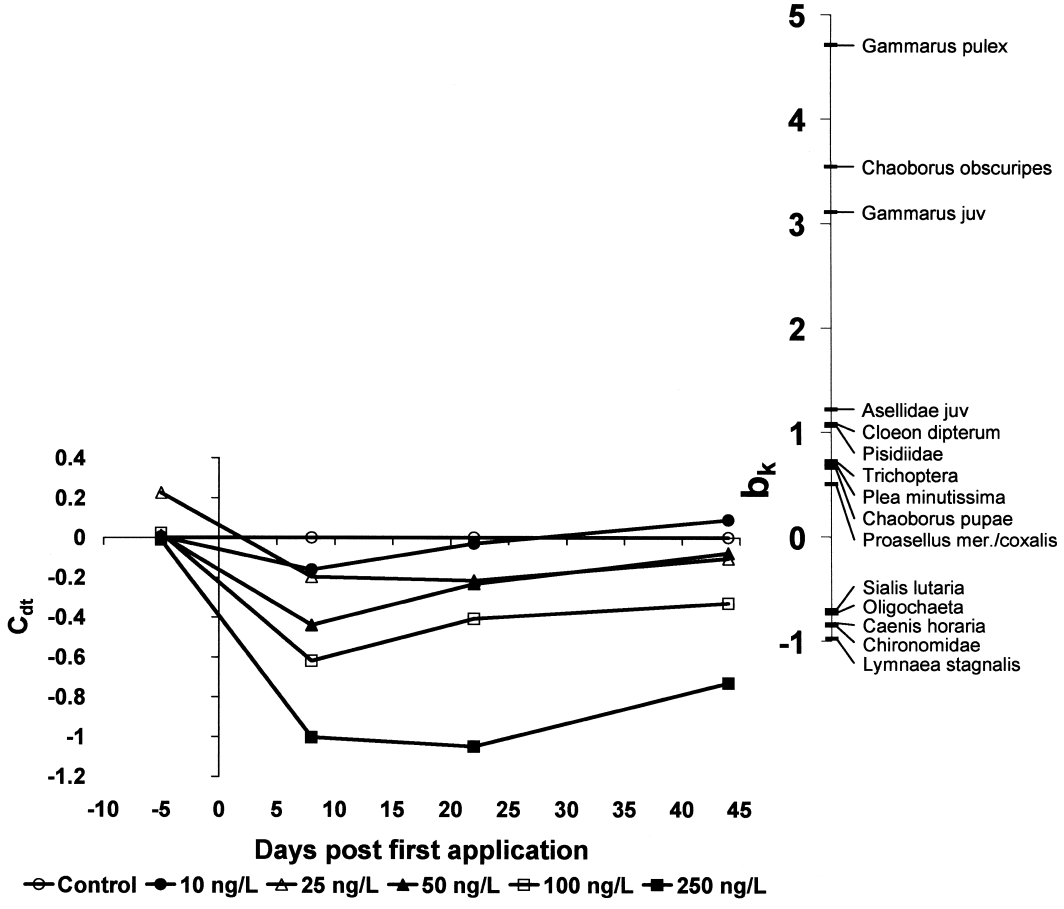

Fig. 2. PRCs indicating effects of spring lambda-cyhalothrin applications on the macroinvertebrate communities in ditch enclosures. Of all variance, 35% could be attributed to sampling date; this is displayed on the horizontal axis. Thirty-five percent of all variance could be attributed to treatment. Of this variance, 29% is displayed on the vertical axis of the PRC diagram. The PRC diagram shows a significant ($p = 0.014$) part of the treatment variance. The vertical axis represents the differences in community structure between treatment and controls expressed as regression coefficients (C_{dt}) of the PRC model. Species weight (b_k) can be interpreted as the affinity of a taxon to the PRC. PRC = principal response curve.

and were most pronounced after these applications (Figs. 2 and 3). NOECs were at their lowest values at 7 days after treatment (<10 ng a.i./L and 10 ng a.i./L for spring and late summer, respectively; Table 4).

In spring, short-term effects occurred down to the 10 ng/L-treatment level. At the 250 ng/L-treatment level, effects were the most pronounced and lasted throughout the study (Fig. 2). Macroinvertebrate communities in enclosures treated with concentrations ≤ 100 ng a.i./L recovered within the study period (Table 4).

In late summer, effects at the 25- and 50-ng/L treatment levels were transient: the two treatment levels were not

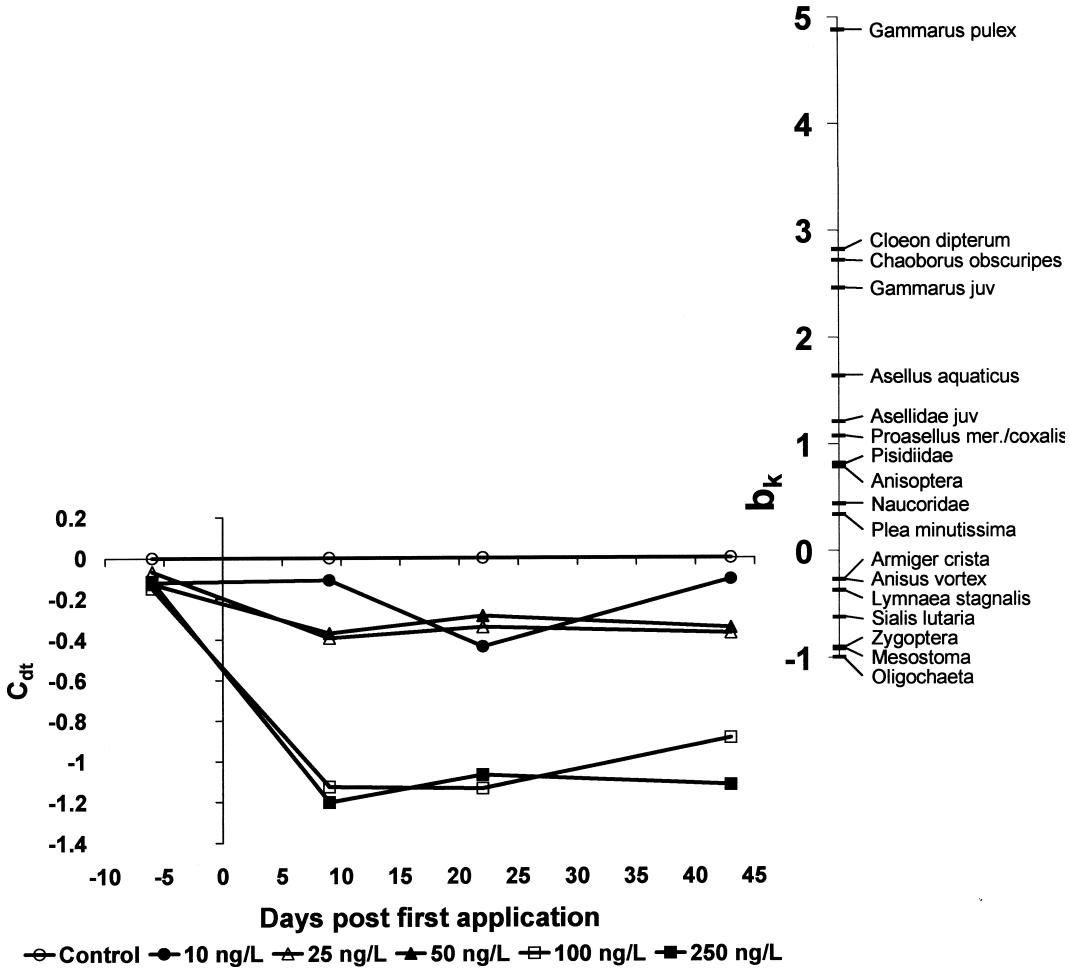

Fig. 3. PRCs indicating effects of late summer lambda-cyhalothrin applications on the macroinvertebrate communities in ditch enclosures. Of all variance, 29% could be attributed to sampling date; this is displayed on the horizontal axis. Forty percent of all variance could be attributed to treatment. Of this variance, 45% is displayed on the vertical axis of the PRC diagram. The PRC diagram shows a significant ($p = 0.008$) part of the treatment variance. The vertical axis represents the differences in community structure between treatment and controls expressed as regression coefficients (C_{dt}) of the PRC model. Species weight (b_k) can be interpreted as the affinity of a taxon to the PRC.

Table 5. Results of Monte Carlo permutation tests (p values) on the combined macroinvertebrate and combined zooplankton data sets of the spring and late summer experiments with lambda-cyhalothrin for testing statistical significance of treatment, differences between seasons, and interaction between "treatment" and "season"

	Macroinvertebrates			Zooplankton		
Week	Treatment	Season	Interaction	Treatment	Season	Interaction
−1	>0.05	0.001	>0.05	>0.05	0.005	>0.05
1	0.001	0.001	>0.05	>0.05	0.005	>0.05
2	–	–	–	>0.05	0.005	>0.05
3	0.001	0.001	>0.05	0.005	0.005	>0.05
4	–	–	–	>0.05	0.005	>0.05
5	–	–	–	>0.05	0.005	>0.05
6	0.002	0.001	>0.05	–	–	–

significantly different from the controls by the second sampling occasion (Week 3; Table 4). The greatest decreases in macroinvertebrate abundance occurred in the 100- and 250-ng/L treatment levels (Fig. 3). Communities exposed to these two treatment regimes did not recover within the study period (Fig. 3 and Table 4).

Although treated macroinvertebrate communities showed consistent statistically significant differences compared with controls, there was no interaction between "treatment" and "season" (Table 5). In other words, the sensitivity of the aquatic community to lambda-cyhalothrin treatment was not significantly different between spring and late summer.

Table 6. NOECs (Williams test $p < 0.05$) per sampling date for macroinvertebrate populations in enclosures exposed to lambda-cyhalothrin in spring and late summer[a]

	NOEC				
Season	−1	1	4	8	See
Spring					
A. crista		100 (↓)			
Asellidae				100 (↓)	
Caenis horaria		100 (↑)			
Chaoborus pupae				10 (↓)	
C. obscuripes		**10 (↓)**	**10 (↓)**		Fig. 5
C. dipterum		100 (↓)			
Gammarus juv.		**100 (↓)**	**100 (↓)**		Fig. 5
G. pulex		**100 (↓)**	**25 (↓)**	**50 (↓)**	Fig. 5
Haliplidae	<10 (↓)				
L. stagnalis			100 (↑)		
Notonecta sp	<10 (↓)				
Sialis lutaria		25 (↑)			
Statistical deviation					
Decrease	2	5	3	3	
Increase	0	2	1	0	
Summer					
A. crista	100 (↓)	100 (↑)			
Asellidae			<10 (↓)		
A. aquaticus		50 (↓)			
C. obscuripes		**<10 (↓)**	**<10 (↓)**	**100 (↓)**	Fig. 6
C. dipterum		25 (↓)		25 (↓)	Fig. 6
Gammarus juveniles		**10 (↓)**	**25 (↓)**		Fig. 6
G. pulex		**50 (↓)**	**50 (↓)**	**50 (↓)**	Fig. 6
Mesostoma spp.	<10 (↓)		100 (↑)		
P. clavata			<10 (↓)		
Sialis lutaria		50 (↑)			
Zygoptera		50 (↑)			
Statistical deviation					
Decrease	2	5	5	3	
Increase	0	3	1	0	

[a] Sampling dates are weeks relative to the first applications. Concentrations (ng a.i./L) greater than NOEC showed significant increases (↑) or decreases (↓). Bolding indicates responses considered consistent, i.e. showing statistical deviations in the same direction for at least two consecutive sampling dates. Number of statistical deviations shows the sum of all NOECs generated per sampling date on the basis of the complete macroinvertebate data set.
NOECs = No observed effect concentrations.

Population Level Response. In spring, consistent responses (*i.e.*, significant responses in the same positive or negative direction for at least two sequential sampling dates) were observed for three taxa, namely *C. obscuripes*, *G. pulex*, and *Gammarus* juveniles (Table 6 and Fig. 4). The number of statistical differences was the highest after the first treatments of lambda-cyhalothrin (Table 6). In addition, significant decreases in the posttreatment period on individual sampling dates were observed in Asellidae, *Chaoborus* pupae, and *C. dipterum* (Table 6). *C. obscuripes* was the species most affected (NOEC 10 ng a.i./L), but it recovered within the study period (Table 6 and Fig. 4). *Gammarus* was affected at the 50 ng/L-treatment and higher (NOEC 25 ng a.i./L). At the 100- and 250-ng/L treatment levels, this species had not recovered at the end of the study period (Table 6 and Fig. 4).

In late summer, responses on at least two sequential sampling dates were again observed for the same three taxa, *C. obscuripes*, *G. pulex*, and *Gammarus* juveniles (Table 6 and Fig. 5). The number of statistical differences was the highest after the first and second treatments of lambda-cyhalothrin (Table 6). Significant decreases in the posttreatment period on individual sampling dates were observed in Asellidae and in *A. aquaticus*, *C. dipterum*, and *Paraponix clavata* (Table 6). Again, *C. obscuripes* (Fig. 5) was the species most affected (NOEC <10 ng a.i./L). Except for the highest treatment level, the species recovered within the study period on the basis of statistical information (Table 6). Abundance, however, was still much lower (≥10-fold) than controls in the 10- to 100-ng/L treatment levels at the end of the study (Fig. 5A), implying a high variability of response among replicates. *Gammarus* juveniles (Fig. 5) were affected at the 25-ng/L treatment and higher (NOEC 10 ng a.i./L). At the end of the experiment, abundances were not significantly different from controls (Table 6). Adult *Gammarus* were significantly affected in the 100- and 250-ng/L treatment levels and had not recovered at the end of the study period (Fig. 5 and Table 6).

Bioassays

Exposure to lambda-cyhalothrin affected the survival of *C. obscuripes*. Calculated EC_{50}s indicated that incipient values

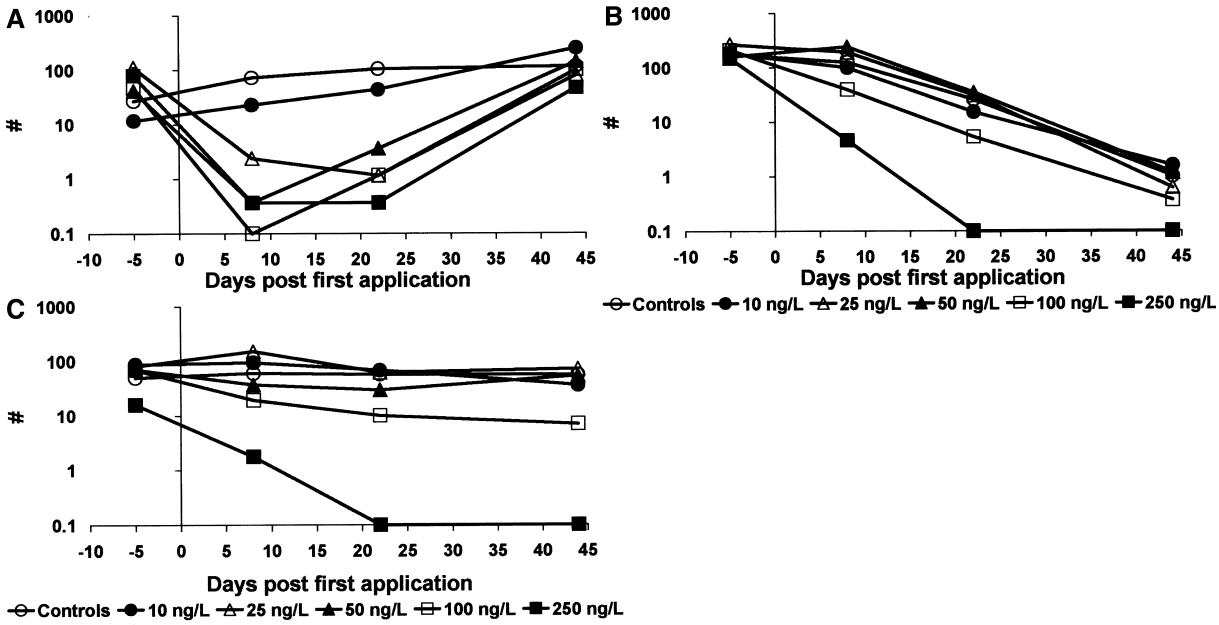

Fig. 4. Dynamics of macroinvertebrate species showing consistent responses in the spring experiment. Geometric mean numbers of (**A**) *Chaoborus obscuripes*, (**B**) *Gammarus* juvenile, and (**C**) *Gammarus pulex*. In each figure part, 0.1 denotes absence.

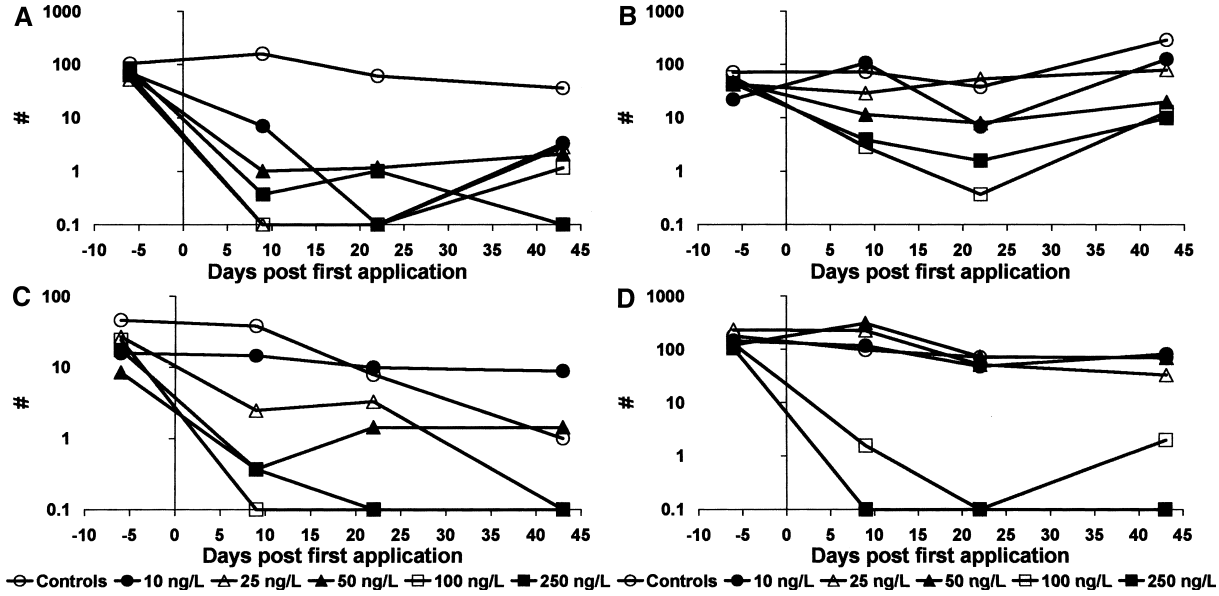

Fig. 5. Dynamics of macroinvertebrate species showing consistent responses in the late summer experiment. Geometric mean numbers of (**A**) *C. obscuripes*, (**B**) *C. dipterum*, (**C**) *Gammarus* juvenile, and (**D**) *G. pulex* are shown. In each figure part, 0.1 denotes absence.

were reached after 2 days (Table 7). The EC_{50} was approximately 5 ng a.i./L in both experiments (Table 7). The response pattern of *A. aquaticus* was similar to that of *C. obscuripes*, in that lambda-cyhalothrin affected the species acutely and responses stabilized within the first 2 days. Incipient EC_{50}s were reached at approximately 70 ng a.i./L in spring and at approximately 50 ng a.i./L in late summer (Table 7). EC_{50} values did not appear to differ significantly between seasons considering the overlapping 95% confidence limits (CIs) (Table 7).

The second series of bioassays, focusing on recovery, showed that for both *Chaoborus* and *Asellus*, EC_{50}, values in spring as well as in late summer increased with time, indicating potential recovery (Table 8). In the case of *A. aquaticus*, bearing in mind the 95% CIs, EC_{50}s did not show consistent differences between spring and late summer (Table 8). For *C. obscuripes*, although 95% CIs overlapped, the EC_{50} values tended to be lower in the late summer bioassay (Table 8). Only in the last bioassay (days 8 through 12) were EC values considerably lower in late summer (Table 8).

Table 7. EC_{50} values (ng a.i./L) obtained from *in situ* cage experiments after the first application in enclosures treated with lambda-cyhalothrin[a]

		EC_{50}	
Test species	Day	Spring	Summer
C. obscuripes	1	14.4 (10.9–19.0)	9.1 (5.5–15.0)
	2	4.9 (2.5–9.9)	5.0 (2.7–9.3)
	3	4.8 (2.2–10.4)	5.2 (2.6–10.3)
	6	8.0[b]	4.3 (1.5–12.8)
A. aquaticus	1	58.0 (46.7–72.0)	70.4 (54.2–91.4)
	2	71.9 (54.5–95.1)	51.9 (40.9–65.8)
	3	69.4 (52.1–92.4)	50.7 (38.5–66.7)
	6	78.9 (56.8–109.5)	48.9 (37.4–64.0)

[a] EC_{50} values are based on nominal concentrations. Lower and upper limits of the 95%-CIs are given between brackets.
[b] No 95% CI limits could be calculated.
CIs = confidence intervals.
EC_{50} = Median effective concentration.

Table 8. EC_{50} values (ng a.i./L) obtained from *in situ* cage experiments to study recovery in enclosures treated with lambda-cyhalothrin[a]

		EC_{50}	
Test species	Day	Spring	Summer
C. obscuripes	0–4	9.6 (6.5–14.4)	7.9 (7.7–8.5)
	4–8	24.9 (21.0–29.6)	17.1 (13.4–21.8)
	8–12	93.0 (79.0–109.4)	28.3 (22.2–36.0)
A. aquaticus	0–4	57.1 (38.3–85.3)	45.3 (39.2–52.3)
	4–8	285.1 (158.6–512.6)	172.5 (144.5–206.0)
	8–12[b]	[c]	313.9 (228.5–431.3)

[a] Lower and upper limits of the 95% CIs are given between brackets.
[b] Eight to 13 days in the late summer experiment.
[c] No EC_{50} could be calculated.
CI = Confidence interval.
EC_{50} = Median effective concentration.

Zooplankton

Community Composition in Spring and Late Summer. A total of 33 and 35 zooplankton taxa were identified in the enclosures during the spring and late summer experiments, respectively. In both experiments, rotifers were the most abundant, followed by cladocerans. The experiments had many taxa in common and several abundant species (*e.g.*, Cyclopoida, nauplii, *Anuraeopsis fissa*) were present in both experiments (Fig. 6). Overall, however, analysis indicated that the late summer zooplankton community structure differed significantly from that in spring (Table 5). The rotifers *Keratella cochlearis*, *Lecane* gr. *lunaris*, *K. quadrata*, *Lepadella patella*, and *Mytilinia ventralis* were less abundant in late summer than in spring (Fig. 6). The taxa in the cluster *Euchlanis dilatata–Trichocerca porcellus* were more abundant in late summer (Fig. 6).

Community Response Spring and Late Summer. Overall, statistical testing did not provide strong evidence of treatment-related effects of lambda-cyhalothrin on the zooplankton communities in both the spring and the late summer experiment (Figs. 7 and 8). In spring, analysis by sampling date yielded an incidental statistically significant deviation for the week 3 sampling (NOEC 25 ng a.i./L; Table 4). In late summer, treatment-related deviations were indicated for three sequential sampling dates (Monte Carlo permutation test). The Williams test, however, did not detect a statistically significant concentration-effect relationship for these same sampling dates (Table 4). No interaction between ''treatment'' and ''season'' could statistically be detected (Table 4). Zooplankton species composition in late summer differed significantly from that in spring (Monte Carlo permutation test $p < 0.05$).

Population Level Response. Univariate analysis of the 33 separate zooplankton populations from the spring experiment resulted in consistent statistically significant responses for four of the taxa. Rotifers (group *A. fissa–T. capucina*) generally tended to increase (Table 9). Copepoda (copepod nauplii and cyclopoida) showed consistent decreases (Fig. 9). NOECs were at the 25-ng/L treatment level (Table 9). As in the macroinvertebrate samples, *C. obscuripes* was most severely affected. Significant decreases of this species were observed in the lowest treatment level (NOEC <10 ng a.i./L). The major decreases in *Chaoborus* populations were observed after the first (week 1) and second (week 2) applications of lambda-cyhalothrin (Fig. 9). Thereafter, only the highest treatment level showed statistically significant decreases

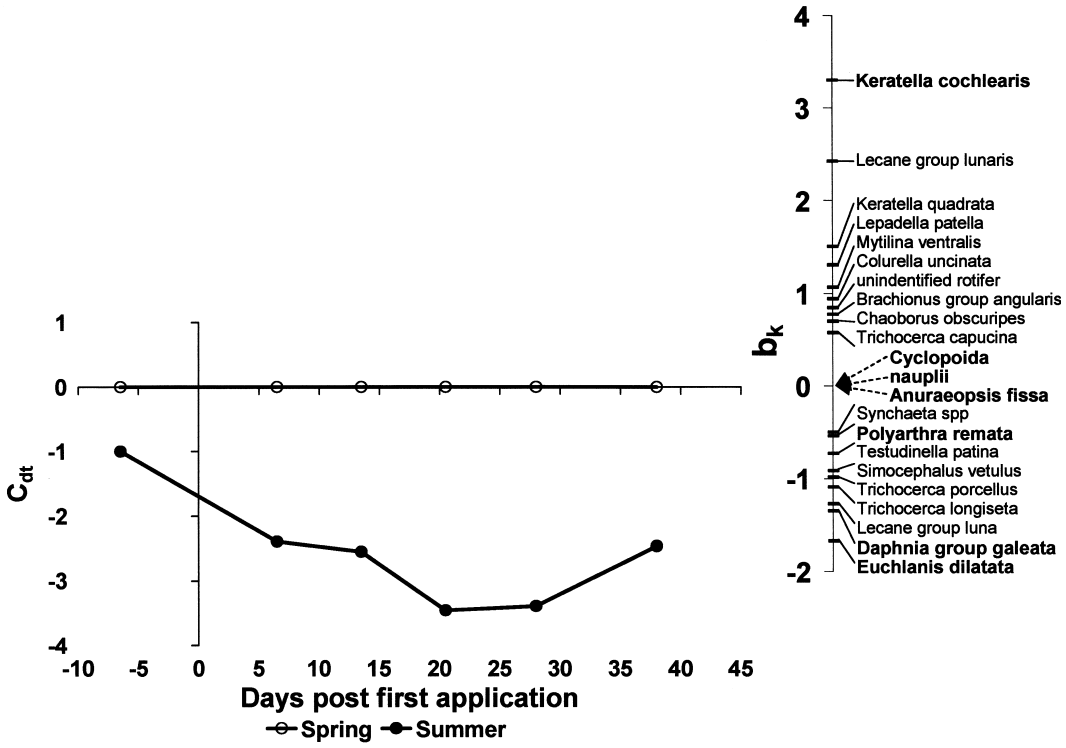

Fig. 6. PRCs indicating the differences in zooplankton species composition in spring and late summer seasons in macrophyte-dominated control enclosures. Of all variance, 23% could be attributed to sampling date; this is displayed on the horizontal axis. Fifty-nine percent of all variance could be attributed to differences between seasons. Of this variance, 65% is displayed on the vertical axis of the PRC diagram. Abundant species are indicated in bold. Abundant species were considered as 25% of all species having the highest abundance numbers per sampling date and were present at that level for three of the four samplings in at least one of both seasons. Species with a weight (b_k) between 0.5 and –0.5 are not shown (except for Cyclopoida, nauplii and *A. fissa*). The vertical axis represents the differences in community structure between treatment and controls expressed as regression coefficients (C_{dt}) of the PRC model. Species weight (b_k) can be interpreted as the affinity of a taxon to the PRC. PRC = principal response curve.

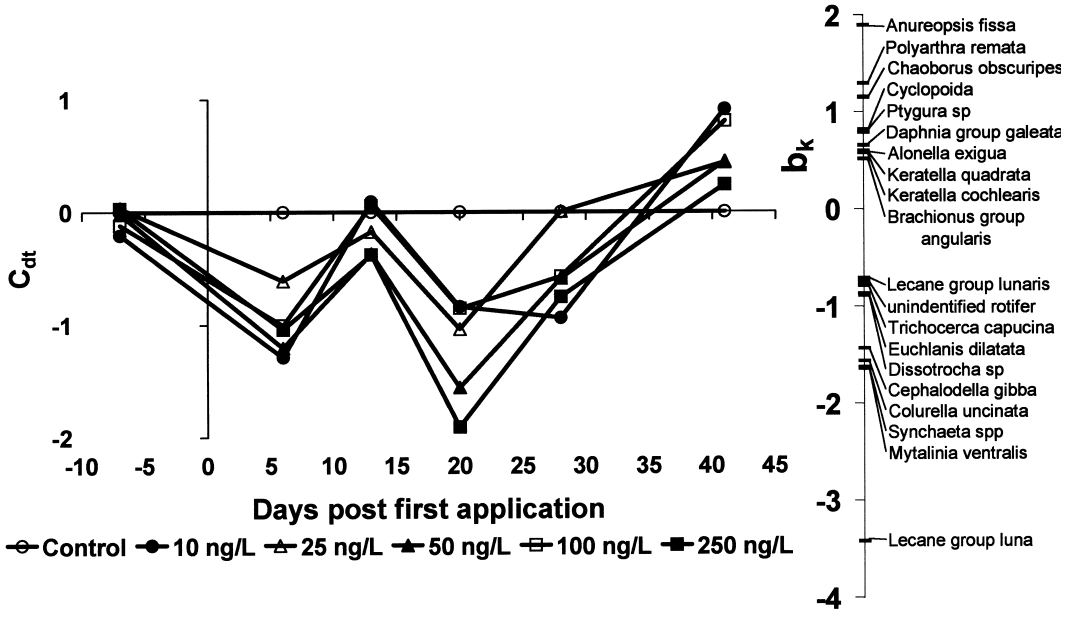

Fig. 7. PRCs indicating effects of spring lambda-cyhalothrin applications on the zooplankton communities in ditch enclosures. Of all variance, 63% could be attributed to sampling date; this is displayed on the horizontal axis. Nineteen percent of all variance could be attributed to treatment. Of this variance, 17% is displayed on the vertical axis of the PRC diagram. The PRC diagram shows a moderately significant (p <0.075) part of the treatment variance. The vertical axis represents the differences in community structure between treatment and controls expressed as regression coefficients (C_{dt}) of the PRC model. Species weight (b_k) can be interpreted as the affinity of a taxon to the PRC. PRC = principal response curve.

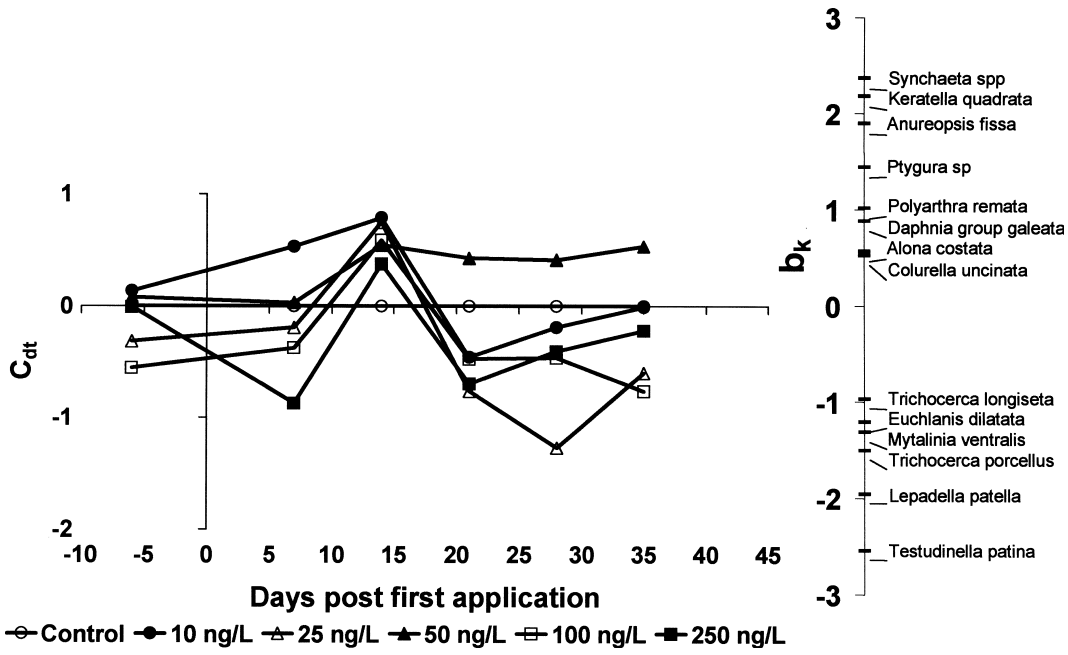

Fig. 8. PRCs indicating effects of the late summer lambda-cyhalothrin applications on the zooplankton community in ditch enclosures. Of all variance, 44% could be attributed to sampling date; this is displayed on the horizontal axis. Twenty-nine percent of all variance could be attributed to treatment. Of this variance, 25% is displayed on the vertical axis of the PRC diagram. The PRC diagram shows a moderately significant ($p < 0.070$) part of the treatment variance. The vertical axis represents the differences in community structure between treatment and controls expressed as regression coefficients (C_{dt}) of the PRC model. Species weight (b_k) can be interpreted as the affinity of a taxon to the PRC. PRC = principal response curve.

(NOEC 100 ng a.i./L) (Table 9 and Fig. 9). Recovery of *Chaoborus* and other species had occurred within the study period (*i.e.*, within 3 weeks after the last treatment) (Table 9).

In late summer, 2 of the 35 zooplankton populations showed consistent responses (Table 9). Unlike the spring experiment, cladocerans (*Daphnia* gr. *galeata*) showed statistically significant decreases at the 100- and 250-ng/L treatment levels. Effects at the 100-ng/L level were observed for 1 week after the third treatment (Fig. 10). Again, *C. obscuripes* was the most sensitive species with significant decreases at the 10-ng/L treatment level. In contrast to the observations in the macroinvertebrate samples, *Chaoborus* had recovered within the study period (compare Tables 6 and 9).

Phytoplankton and Periphyton

Neither in spring nor in late summer did chlorophyll-*a* concentrations for either phytoplankton or periphyton show treatment-related effects. Only once, in the fourth week of the posttreatment period of the spring experiment, were phytoplankton chlorophyll-*a* concentrations significantly lower than control levels (mean control level 50 μg/L versus 20 to 33 μg/L in the treated systems; Williams test $p < 0.05$). In contrast, chlorophyll-*a* amounts for both the phytoplankton and periphyton were lower in late summer than in spring. Phytoplankton chlorophyll-*a* concentrations during the entire experimental period in the controls of the spring experiment were 42 ± 11 μg/L compared with 12 ± 5 μg/L (mean ± SD) in late summer. Similarly, mean periphyton chlorophyll-*a* amounts were 32 ± 29 μg/m^2 and 5 ± 2 μg/m^2 in spring and late summer, respectively.

Macrophytes

In spring, mean macrophyte biomass surrounding the enclosures increased with time (104 ± 35 to 138 ± 16 g/m^2 dry weight [dw]), indicating that the vegetation was in a growth phase (Fig. 11A). In late summer, this biomass showed a decrease with time (241 ± 108 to 167 ± 10 g/m^2 dw), indicating that the vegetation was in its decline phase (Fig. 11A). In both experiments, vegetation harvested in the enclosures at the end of the experiments did not statistically differ (Williams test $p > 0.05$) between treatment levels (Fig. 11B).

Decomposition and Community Metabolism

In spring, no effects on the decomposition of *Populus* leaf litter were measured. In late summer, the remaining biomass of *Populus* leaves was significantly higher in the 100- and/or 250-ng/L treatment levels than in the controls (Williams test $p < 0.05$) (Table 10). Overall, the impact on community metabolism end points was not very pronounced. Concentrations of DO and pH in treated enclosures did not differ significantly from the controls in either experiment (Table 11). Conductivity differed significantly from the controls in spring

Table 9. NOECs (Williams test $p < 0.05$) per sampling date for zooplankton populations in enclosures exposed to lambda-cyhalothrin in spring and late summer[a]

	NOEC							
Season	−1	1	2	3	4	5	6	See
Spring								
A. fissa				100 (↓)				
Brachionus spp.	<10 (↑)							
B. anguilaris					<10 (↓)			
Cephalodella gibba		100 (↑)						
Colunaris uncinata		100 (↑)						
Lecane gr. *lunaris*			**100 (↑)**	**<10 (↑)**				Fig. 10
Lecane gr. *luna*		<10 (↑)						
Synchaeta spp					100 (↑)			
T. capucina				50 (↑)				
Nauplii		**100 (↓)**	**25 (↓)**	**100 (↓)**				Fig. 10
Cyclopoida				**25 (↓)**	**100 (↓)**			Fig. 10
Daphnia gr. *galeata*					10 (↓)			
Ostracoda	<10 (↓)							
C. obscuripes		**10 (↓)**	**<10 (↓)**	**100 (↓)**	**100 (↓)**			Fig. 10
Statistical deviation								
Decrease	1	2	2	4	4	–	0	
Increase	1	3	1	1	1		0	
Summer								
Synchaeta spp.			<10 (↑)			<10 (↑)		
Nauplii				100 (↓)		100 (↓)		
Daphnia gr. *galeata*		**100 (↓)**	**100 (↓)**	**50 (↓)**	**100 (↓)**			Fig. 11
Ostracoda					100 (↑)			
C. obscuripes		**<10 (↓)**	**<10 (↓)**		<10 (↓)			Fig. 11
Statistical deviation								
Decrease	0	2	2	2	2	1	–	
Increase	0	0	1	0	1	1	–	

[a] Sampling dates are weeks relative to the first applications. Concentrations (ng a.i./L) greater than NOEC showed significant increases (↑) or decreases (↓). Bolded text indicates responses considered consistent, i.e., showing statistical deviations in the same direction for at least two consecutive sampling dates. Number of statistical deviations shows the sum of all NOECs generated per sampling date on the basis of the complete macroinvertebate data set.
NOEC = No observed effect concentration.

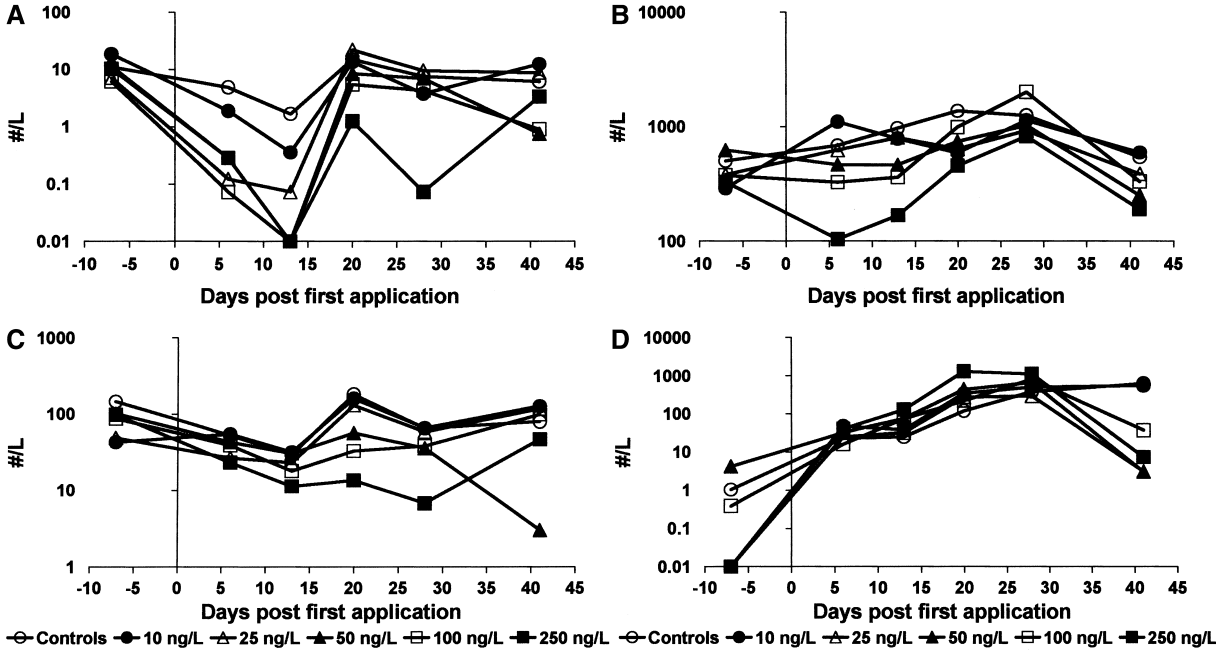

Fig. 9. Dynamics of zooplankton species showing consistent responses in the spring experiment. Geometric mean numbers of (**A**) *C. obscuripes*, (**B**) nauplii, (**C**) Cyclopoida, and (**D**) *Lecane* group *lunaris* are shown. In the figures, 0.01 denotes absence.

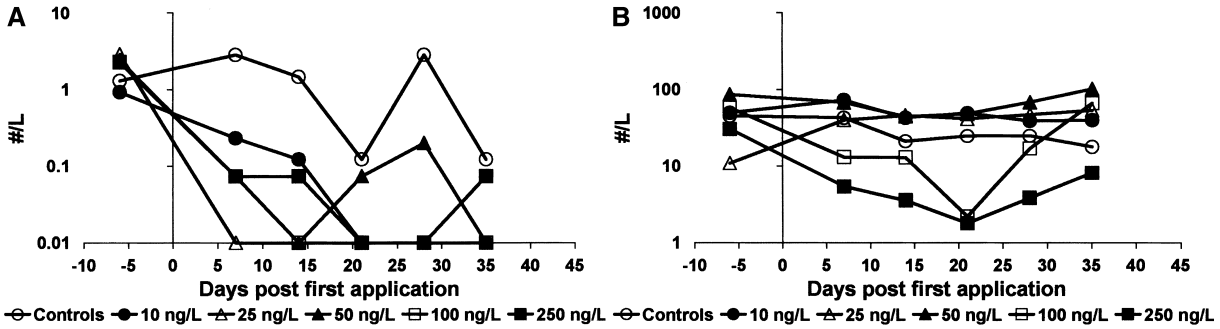

Fig. 10. Dynamics of zooplankton species showing consistent responses in the late summer experiment. Geometric mean numbers of (**A**) *C. obscuripes* and (**B**) *Daphnia* group *galeata* are shown. In figure part A, 0.01 denotes absence.

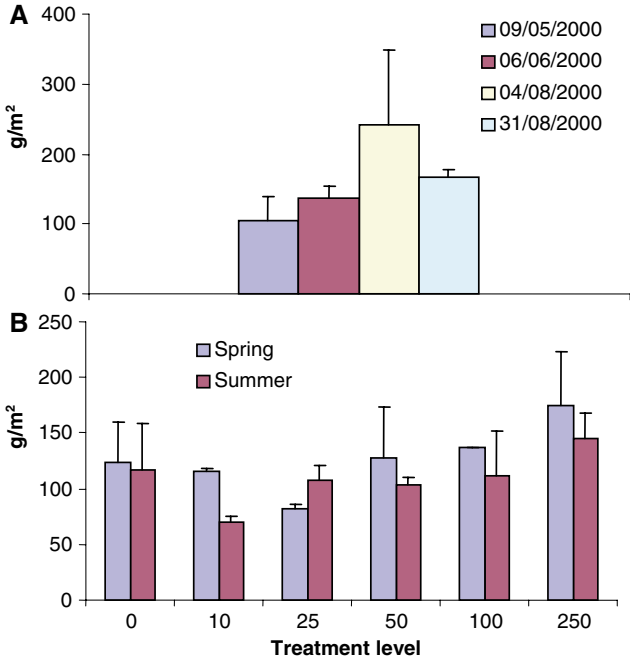

Fig. 11. Mean dry weight of macrophytes (g/m^2) collected in ditches housing the enclosures for the spring and late summer experiments. Macrophytes were collected outside enclosures (**A**) and inside enclosures at the end of the experiments (**B**) No statistically significant differences between treatments and controls were detected (Williams test $p > 0.05$). Williams tests were done on the enclosure vegetations of the spring and summer experiments.

at the treatment levels ≥50 ng/L, but only in the last sampling week (Table 11). Only in the 250-ng/L treatment level did alkalinity show consistent deviations in spring. Differences, however, between controls and treated systems were small (*e.g.*, week 3 mean controls values were 1.06 meq/L and the treated systems values were between 0.91 and 0.98 meq/L). In late summer, these two end points did not differ statistically (Table 11). DO and pH were higher, and conductivity and alkalinity were lower in spring than in late summer (Table 1). This pattern of the DO–pH–conductivity–alkalinity syndrome indicated that there was higher photosynthetic activity in the spring experiment, as would be expected.

Discussion

Experimental Design

We compared the impact of lambda-cyhalothrin in 1 experimental ditch in spring with that of another experimental ditch in late summer. This means that at the level of the ditch, observations were not replicated. Strictly speaking, this implies that differences detected between experiments cannot be attributed solely to "ditch" or "season." However, both ditches were from the same population of 12 similar ditches with shared history, climate, and biologic communities since their construction in the late 1980s. This makes it reasonable to conclude that any differences in impact between the experiments are a result of the factor "season" and not of the factor "ditch."

Fate

Because lambda-cyhalothrin is highly lipophilic (log Kow = 7), the compound tends to bind greatly and rapidly to nonaquatic surfaces such as organic materials and sediment (Hand *et al.* 2001). The high spatial variation in concentrations in the water found shortly after the applications indicated that the distribution of the compound between the different compartments was still in progress. It is well known for pyrethroids that exposure concentrations are difficult to measure in the highly dynamic phase shortly after application. In our study, substantial dissipation of the compound had occurred within the first day of treatment (Leistra *et al.* 2003).The rapid decrease in water concentrations of lambda-cyhalothrin was expected because this has been reported previously in many studies with synthetic pyrethroids (*e.g.*, Hill 1989; Hand *et al.* 2001).

The three applications of lambda-cyhalothrin did not lead to an accumulation of the compound in the water phase. Concentrations were very low and/or at the detection limit level by 7 days (Leistra *et al.* 2003), at which point the next application was made. The rapid dissipation of lambda-cyhalothrin indicates that during the study the communities were subjected to repeated, short-term exposures rather than chronic exposures.

Table 10. Remaining dry weight of *Populus* leaves in litterbags[a] Initial *Populus* dry weight was 2 g

		g dw					
Season	Week	Controls	10	25	50	100	250
Spring							
	−3	1.34	1.31	1.18	1.30	1.36	1.38
	−1	1.36	1.32	1.32	1.30	1.36	1.38
	1	1.41	1.36	1.38	1.42	1.45	1.46
	4	1.38	1.37	1.32	1.41	1.41	1.40
Summer							
	−3	1.23	1.15	1.27	1.09	1.20	1.23
	−1	1.20	1.10	1.23	1.18	1.23	1.24
	1	1.33	1.33	1.40	1.39	1.40[b]	1.42[b]
	4	1.33	1.33	1.36	1.34	1.39	1.41[b]

[a] Duration of experiments was 2 weeks. Mean values (g dw) per treatment level of lambda-cyhalothrin (ng/L) are given. One series of experiments was performed in spring and one in late summer.
[b] Values deviated significantly from control levels (Williams test, $p < 0.05$).

Table 11. NOECs (Williams test $p < 0.05$) per sampling date for community metabolism end points in enclosures treated with lambda-cyhalothrin (treatments: 0 to 250 ng/L)[a]

	NOEC					
Season Week	−1	1	2	3	4	5
Spring						
DO[b]						
pH[b]						
EC						25 (↓)
Alkalinity				< 10 (↓)	100 (↓)	25 (↓)
Summer[b]						

[a] One experiment was performed in spring and one in late summer. End points were EC, DO, pH, and alkalinity. Treatments resulted in significant decreases (↓) (Williams test $p < 0.05$).
[b] No statistically significant deviations found.
DO = Dissolved oxygen.
EC = Electrical conductivity.
NOEC = No observed–effect concentration.

Acute Effects

As would be expected from laboratory and other field toxicity data (Schroer *et al.* 2004; Maund *et al.* 1998), lambda-cyhalothrin applications resulted in effects down to and including the 10-ng/L treatment level and specifically occurred within arthropod populations. Observations at the water surface indicated that clear effects, ranging from agitated to dead specimens, had already developed within 10 hours after application of lambda-cyhalothrin. This rapid onset of effect coupled with rapid dissipation from the water column is typical for synthetic pyrethroids. In both experiments, the number of statistical significant effects clearly increased after the treatments started. For the most sensitive group, the arthropods, effects were greatest immediately directly after the first application (Table 6), suggesting that the compound had its major impact on this group after the first applications.

Secondary Effects

Although some decreases in the zooplankton were observed at the higher treatment levels, these did not lead to indirect effects in the form of increases of the algae because of a release of grazing pressure. Functional redundancy might have dampened secondary responses because relatively complex natural species assemblages were present. Algal development might also have been repressed because of the dominance of macrophytes in the test systems. Lambda-cyhalothrin treatments did not or only had minor effects on the community metabolism parameters measured. Later in the experiment, decomposition was decreased in late summer at the 100- and 250-ng/L treatment levels, which may be explained by the decrease of sensitive macroinvertebrate shredders (*e.g.*, *G. pulex*, *A. aquaticus*). It may be that the effect observed in late summer was obscured in spring because availability of suitable organic matter in the detritus layer is greater in spring. Compared with late summer, this could have resulted in less need for the food source in the litter bags. Inherently, the smaller differences between presence and absence of consumption by shredders are harder to detect.

Roessink *et al.* 2005 noted that in spring there was a tendency for higher densities of cladocerans in the enclosures treated with 25, 50, and 100 ng a.i./L compared with those in the controls and the 250-ng/L treatment level. The investigators explained this response pattern as a combination of direct toxic and indirect effects. The relatively low abundance of cladocerans in the controls was probably the result of high predation pressure by *Chaoborus*. With increasing treatment levels up to 100 ng a.i./L, predation by *Chaoborus* decreases, resulting in higher densities of cladocerans, which are less sensitive to lambda-cyhalothrin. This similar inverted U-shaped response also seems to have occurred later in the season because the abundance of cladocerans in the intermediate treatment levels of 10 to 50 ng a.i./L tended to be higher than in the controls and the 100– and 250 ng a.i./L–treated enclosures of the late summer experiment (see Fig. 10B as an example).

Comparison of Seasons

The communities of the two macrophyte-dominated test systems did not differ very much in macrophyte structure and biomass (Fig. 11B). The amount of algae was lower in the late summer

Table 12. Summary of effects observed in the spring and summer experiments in enclosures treated with lambda-cyhalothrin[a]

	Treatment levels (ng/L)				
Season End point	10	25	50	100	250
Spring					
PRC macroinvert	2	2	3	3	4
Macrocrustaceans	1	2↓	2↓	4↓	4↓
Insecta (excl. *Chaob*)	1–2↓	3↓	3↓	3↓	3↓
C. obscuripes	2↓	3↓	3↓	3↓	3↓
Remaining macroinvertebrates	1	1	1	1	2↓↑
PRC zooplankton	1	1	2	2	2
Microcrustaceans	1	2↓	2↓	2↓	4↓
Rotifers	2↓↑	2↓↑	2↓↑	2↓↑	2↓; 3↑
Algae	1	1	1	1	1
Macrophytes	1	1	1	1	1
Community metabolism	1	1	1	1	1
Summer					
PRC macroinvert	1	2	2	4	4
Macrocrustaceans	1	2↓	3↓	4↓	4↓
Insecta (excl. *Chaob*)	1–2↓	1–2↓	4↓	4↓	4↓
C. obscuripes	3↓	3↓	3↓	3↓	4↓
Remaining macroinvertebrates	1	1	1	1	2↑
PRC zooplankton	1	1	1	1	1
Microcrustaceans	1	1	1	2↓	3↓
Rotifers	2↑	2↑	2↑	2↑	2↑
Algae	1	1	1	1	1
Macrophytes	1	1	1	1	1
Community metabolism	1	1	1	1	1

[a] The numbers in the table follow the effect classes as described by Brock *et al.* (2000). 1 = no effect; 2 = slight effects; 3 = clear short-term effects, full recovery observed (within 4 to 8 weeks); 4 = clear effects, no full recovery observed at the end of the experiment. ↓ = decreased end point; ↑ = increased end point; ↓↑ decreased and increased end point.
Excl. *Chaob* = without *C. obscuripes*.
PRC = principle response curves of either macroinvertebrates or zooplankton.

experiment. This phenomenon can be explained by seasonal shifts in algal abundances because of competition on nutrients between algae and the dominating macrophytes (Scheffer 1998). Also, compared with spring, community metabolism was lower in late summer. Despite the statistically significant differences in species composition between both experiments, communities varied little with respect to dominant and sensitive species (*e.g.*, *Chaoborus* and *Gammarus*; Fig. 1).

Summarizing the two experiments into effect classes shows that, except for *Chaoborus*, only slight and transient effects were observed at the lowest treatment level in both experiments (Table 12). In spring, this was expressed at the community level and concerned only some populations, whereas in the late summer only some populations of sensitive macrocrustaceans and insects showed incidental negative responses at the 10-ng/L treatment level. In combination with the analysis of interactions between ''season'' and ''treatment'' at the community level, which indicated that there was no significant interaction between these two factors, our study suggests that sensitivity of the macrophyte-dominated system was independent of season. Considering the inconsistent and few incidental responses at the 10-ng/L treatment level on both the community as well as on population level, the $NOEC_{community}$ is lower but near to this treatment level in both experiments. At higher concentration levels, the overall picture shows that clear effects tend to be of shorter duration in spring than in late summer.

The only exception of the general finding of approximately similar threshold levels for both seasons was the response of *C. obscuripes*. In late summer, this species showed clear effects followed by recovery (effect class 3) at the 10-ng/L treatment level, whereas it only showed slight effects (effect class 2) in spring (Table 12). One difference in effect was that initial decreases were larger in late summer than in spring (compare, *e.g.*, Figs. 4A and 5A). To find out whether this difference in effects could be explained by differences in the relative contributions of older and younger cohorts in the populations of *Chaoborus*, the head lengths of specimens caught in the zooplankton control samples of the first sampling posttreatment were measured (according to Swift and Federenko 1975). We observed that the spring population was dominated by individuals in younger life stages, whereas the late summer population was dominated by older ones. Assuming that individuals in younger life stages are more sensitive, as is often found (Hutchinson *et al.* 1998; Stark 1999), it appears that differences in cohort structure of the *Chaoborus* populations do not explain the more severe decreases in late summer.

We also investigated whether small differences in exposure concentrations could have had an influence. Mean nominal initial concentrations in spring were 9.5 ng a.i./L compared with 10.5 a.i./L in late summer at the lowest treatment level (Table 3). Based on a laboratory concentration–response relationship for *C. obscuripes*, the affected fraction in this concentration range would be 85% to 86% (after Schroer *et al.*

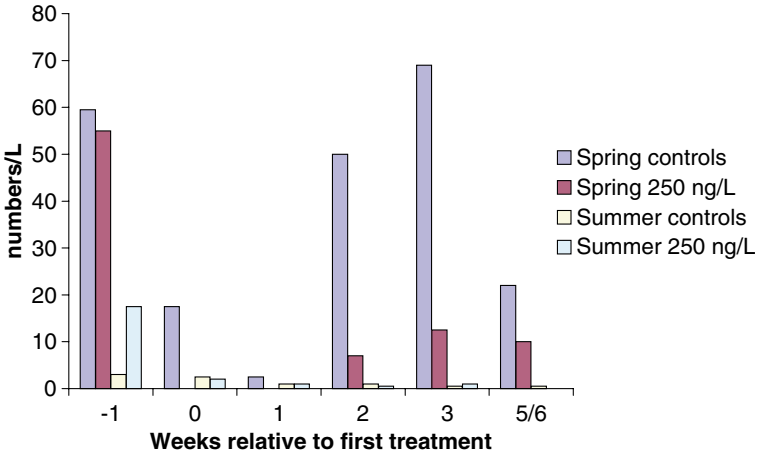

Fig. 12. Mean numbers of specimens within the youngest life-stage class of *C. obscuripes* in relation to time. Youngest life-stage class contained larvae with head lengths ≤ 0.6 mm. Applications of lambda-cyhalothrin were in done in weeks 0 to 2.

2004). Another possibility causing differences between the responses might be that differences in bioavailability of lambda-cyhalothrin between the experiments might have occurred, for instance, because of the higher phytoplankton densities in spring. The similarity in response of *Chaoborus* in the *in situ* bioassays, however, also make this possibility ambiguous.

The approach used here to categorize ''severity of effects'' includes both inherent ''sensitivity'' along with the duration of the observed effects. The time span of effects on *Chaoborus* was longer in late summer, when recovery did not occur, or was not complete (compare Figs. 4A and 5A). However, the *in situ* cage experiments with *Chaoborus* after the last treatment demonstrated that in both experiments, recovery of the species potentially was possible shortly after the treatments (significant increase in toxicity values within 8 to 12 days after the last treatment [Table 8]).

The differences in actual recovery can be explained by the different recolonization patterns found for spring and late summer. The number of specimens in the youngest life-stage class found in the zooplankton samples indicate that the colonization rate of *Chaoborus* is much higher in spring than in late summer. In spring, we found several tens of new recruits at every sampling date. In late summer, these numbers were well below 10 individuals (Fig. 12). Recolonization, even at the highest treatment level, started in the week of the last spring application. In late summer, recolonization hardly occurred at all (Fig. 12).

This difference in recolonization potential of *Chaoborus* had a considerable impact on the recovery of the complete macroinvertebrate community, as indicated in the PRC analysis (Figs. 2 and 3). Together with *Gammarus*, *Chaoborus* dominated the community response curves because they have the highest species weights. *Gammarus*, however, was a constant factor in the sense that it was eradicated at the highest treatment level in both experiments, and in neither case could it recover due to lack of suitable recolonization conditions because this species is obligately aquatic. The dominant varying factor was the difference in abundance of *Chaoborus*, and thus this contributed greatly to the overall community response. Lack of recovery of the community in late summer was mainly related to the absence of recolonization by *Chaoborus* later in the season.

Comparison With Other Studies

The partner study, which focused on differences in effects between macrophyte- and plankton-dominated systems, indicated that the factors ''treatment regime'' and ''community structure,'' as well as the interaction between the two, were statistically significant variables. This indicated that, overall, the macrophyte- and plankton-dominated systems responded differently to the same treatment regime although the overall threshold levels were similar (Roessink *et al.* 2005). In the plankton-dominated systems only slight and transient effects at the 10-ng/L treatment level were observed. An indoor microcosm study with a pesticide mixture containing several applications of lambda-cyhalothrin, gave a $NOEC_{community}$ at the treatment level containing 10 ng lambda-cyhalothrin/L. However, lack of response at the 10-ng/L treatment level was explained by the very low numbers of *C. obscuripes* in the test systems (Van Wijngaarden *et al.* 2004). Microcosm studies have indicated that pronounced effects (effect classes 3 to 5) of lambda-cyhalothrin on sensitive populations can be expected at exposure concentrations of 16 to 25 ng a.i./L and higher (Hill *et al.* 1994; Farmer *et al.* 1995; Roessink *et al.* 2005; present study).

Overall, our study did not provide straightforward evidence of major differences in effects around threshold levels between spring and late summer. At higher concentrations, recovery took more time in late summer. Similar observations have been reported for an enclosure experiment with pentachlorophenol (Willis *et al.* 2004).

For temperate regions, the CLASSIC guidance document (Community-Level Aquatic Systems Studies Interpretation Criteria) which deals with the interpretation of results of aquatic microcosm and mesocosm studies in relation to risk-assessment procedures of pesticides, recommends applying test substances in the period between spring and midsummer (Giddings *et al.* 2002). On the basis of outdoor model ecosystem experiments (Willis *et al.* 2004; present study), it seems that exposure concentrations around threshold levels for direct effects observed in early-season studies are reasonably predictive for effects later in the season. Above these threshold concentrations, however, severity, duration, and type of direct as well as indirect effects may vary much more during

different periods of the year because of seasonal variation in population densities, recovery potential, and food web interactions.

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References

Campbell PJ, Arnold DJS, Brock TCM, Grandy NJ, Heger W, Heimbach F, et al. (1999) Guidance document on higher tier aquatic risk assessment for pesticides (HARAP). Society of Environmental Toxicology and Chemistry Europe, Brussels, Belgium

Brock TCM, Crum SJH, Van Wijngaarden RPA, Budde BJ, Tijink J, Zupelli A, et al. (1992) Fate and effects of the insecticide Dursban® 4E in indoor *Elodea*-dominated and macrophyte-free freshwater model ecosystems: I. Fate and primary effects of the active ingredient chlorpyrifos. Arch Environ Contam Toxicol 23:69–84

Bérard A, Pelte T, Druart J-C (1999) Seasonal variations in the sensitivity of Lake Geneva phytoplankton community structure to atrazine. Arch Hydrobiol 145:277–295

Farmer D, Hill IR, Maund SJ (1995) A comparison of the fate and effects of two pyrethroid insecticides (lambda-cyhalothrin and cypermethrin) in pond mesocosms. Ecotoxicology 4:219–244

Giddings JM (1982) Effects of the water-soluble fraction of a coal-derived oil on pond microcosms. Arch Environ Contam Toxicol 11:735–747

Giddings JM, Brock TCM, Heger W, Heimbach F, Maund SJ, Norman SM, et al. (eds) (2002) Community-level aquatic systems studies—Interpretation criteria. Proceedings from the CLASSIC Workshop (Society of Environmental Toxicology and Chemistry publication), Schmallenberg, Germany, May 30 to June 2, 1999

Hanazato T, Yasuno M (1990) Influence of time of application of an insecticide on recovery patterns of a zooplankton community in experimental ponds. Arch Environ Contam Toxicol 19:77–83

Hand LH, Kuet SF, Lane MCG, Maund SJ, Warinton JS, Hill IR (2001) Influences of aquatic plants on the fate of the pyrethroid insecticide lambda-cyhalothrin in aquatic environments. Environ Toxicol Chem 20:1740–1745

Hill IR (1989) Aquatic organisms and pyrethroids. Pestic Sci 27:429–465

Hill IR, Runnalls JK, Kennedy JH, Ekoniak P (1994) Lambda-cyhalothrin: A mesocosm study of its effects on aquatic organisms. In: Graney RL, Kennedy JH, Rogers JH (eds) Aquatic mesocosm studies in ecological risk assessment (special publication of the Society of Environmental Toxicity and Chemistry). Lewis, Michigan, pp 403–467

Hommen U, Veith D, Ratte H-T (1994) A computer program to evaluate plankton data from freshwater field tests. In: Hill IA, Heimbach F, Leeuwangh P, Matthiesen P (eds) Freshwater field tests for hazard assessment of chemicals. Lewis, Boca Raton, FL, pp 503–513

Hutchinson TH, Solbé J, Kloepper-Sams PJ (1998) Analysis of the ECETOC aquatic toxicity (EAT) database. III–Comparative toxicity of chemical substances to different life stages of aquatic organisms. Chemosphere 36:129–142

Kindig AC, Conquest LL, Taub FB (1983) Differential sensitivity of new versus mature synthetic microcosms to streptomycin sulfate treatment. Aquatic Toxicology and Hazard Assessment: Sixth symposium, American Society for Testing and Materials STP 802. In: Bishop WE, Cardwell RD, Heidolph (eds). American Society for Testing and Materials, Philadelphia, PA, pp 192–203

Leistra M, Zweers AJ, Warington JS, Crum SJ, Hand LH, Beltman WHJ, et al. (2003) Fate of the insecticide lambda-cyhalothrin in ditch enclosures differing in vegetation density. Pest Manage Sci 60:75–84

Mayer FL, Ellersieck MR (1986) Manual of acute toxicity: Interpretation and database for 410 chemicals and 66 species of freshwater animals. United States Fish and Wildlife Service Resource Publication 160. United States Department of the Interior, Washington, DC

Maund SJ, Hamer MJ, Warinton JS, Kedwards TJ (1998) Aquatic ecotoxicology of the pyrethroid insecticide lambda-cyhalothrin: Considerations for higher-tier aquatic risk assessment. Pestic Sci 54:408–417

Moed JR, Hallegraeff GM (1978) Some problems in the estimation of chlorophyll- *a* and phaeopigments from pre- and post-acidification spectrophotometric measurements. Int Rev Gesamten Hydrobiol 63:787–800

Roessink I, Arts GHP, Belgers JDM, Bransen F, Maund SJ, Brock TCM (2005). Effects of lambda-cyhalothrin in two ditch microcosm systems of different trophic status. Environ Toxicol Chem 24:1684–1696

SANCO (2002) Guidance document on aquatic ecotoxicology in the context of the directive 91/414/EEC, working document SANCO/3268/2001 revision 4 (final), 2002. European Commission, Health and Consumer Protection Directorate–General

Scheffer M (1998) Ecology of shallow lakes. Chapman and Hall, London, UK

Schroer AFW, Belgers D, Brock TCM, Matser A, Maund SJ, Van den Brink PJ (2004) Comparison of laboratory single species and field population-level effects of the pyrethroid insecticide *lambda*-cyhalothrin on freshwater invertebrates. Arch Environ Contam Toxicol 46:324–335

Stark JD (1999) Population structure and differential susceptibility among life stages: Implications for population effects of toxicants. Asp Appl Biol 53:235–240

Swartzman GL, Taub FB, Meador J, Huang C, Kindig A (1990) Modeling the effect of algal biomass on multispecies aquatic microcosms response to copper toxicity. Aquat Toxicol 17:93–118

Swift MC, Fedorenko AY (1975) Some aspects of prey capture by *Chaoborus larvae*. Limnol Oceanogr 20:418–425

Taub FB, Kindig AC, Meador JP, Swartzman GL (1991) Effects of "seasonal succession" and grazing on copper toxicity in aquatic microcosms. Verh Internat Verein Limnol 24:2205–2214

Ter Braak CJF, Smilauer P (1998) CANOCO reference manual and user's guide to CANOCO for Windows: Software for canocical community ordination (version 4). Microcomputer Power, Ithaca, NY

Van den Brink PJ, Hattink J, Bransen F, Van Donk E, Brock TCM (2000) Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. Aquat Toxicol 48:251–264

Van den Brink PJ, Ter Braak CJF (1998) Multivariate analysis of stress in experimental ecosystems by principal response curves and similarity analysis. Aquat Ecol 32:163–178

Van den Brink PJ, Ter Braak CJF (1999) Principal response curves: Analysis of time-dependent multivariate responses of a biological community to stress. Environ Toxicol Chem 18:138–148

Van den Brink PJ, van Wijngaarden RPA, Lucassen WGH, Brock TCM, Leeuwangh P (1996) Effects of the insecticide Durban® 4E (a.i. chlorpyrifos) in outdoor experimental ditches: II. Community responses and recovery. Environ Toxicol Chem 15:1143–1153

Van Wijngaarden RPA, Cuppen JGM, Arts GHP, Crum SJH, Van den Hoorn MW, Van den Brink PJ, Brock TCM (2004) Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: A microcosm study. Environ Toxicol Chem 23:1479–1498

Van Wijngaarden RPA, Brock TCM, Douglas M 2005. Effects of chlorpyrifos in freshwater model ecosystems: The influence of experimental conditions on ecotoxicological thresholds. Pest Manage Sci 64:923–935

Williams DA (1972) The comparison of several dose levels with a zero dose control. Biometrics 28:519–531

Willis KJ, Van den Brink PJ, Green JD (2004) Seasonal variation in plankton community responses of mesocosms dosed with pentachlorophenol. Ecotoxicology 13:707–720