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Effects of the pyrethroid insecticide gamma-cyhalothrin on aquatic invertebrates in laboratory and outdoor microcosm tests

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Abstract The sensitivity of a range of freshwater lentic invertebrates to gamma-cyhalothrin (GCH), a single enantiomer of the synthetic pyrethroid lambda-cyhalothrin, was assessed in single species laboratory tests and an outdoor multi-species ecosystem test. The most sensitive species in the laboratory single species tests with GCH was *Chaoborus obscuripes* (96 h EC_{50}: 3.8 ng/l). The species sensitivity distribution curve, based on the laboratory 96 h EC_{50} acute toxicity data for eight species, gave a median HC_5 value for GCH of 2.12 ng/l. The $NOEC_{community}$ derived from the multi-species ecosystem test was 5 ng/l, and the insects *Chaoborus* sp. and *Caenis* sp. were identified as the most sensitive species. The results indicate that the median HC_5, based on eight species selected to include those known to be sensitive to pyrethroids, provided a good estimation of the $NOEC_{community}$ for GCH. Furthermore, the results for GCH indicated that the endpoints typically used in higher-tier risk assessments for pesticides in Europe (HC_5 and $NOEC_{community}$) were consistent with expectations when compared to the equivalent endpoints for the racemate LCH.

Keywords Pyrethroid · Gamma-cyhalothrin · Species sensitivity distribution · Aquatic microcosm

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Introduction

Gamma-cyhalothrin (GCH) is a single, resolved enantiomer of the synthetic pyrethroids cyhalothrin (CH) and lambda-cyhalothrin (LCH), and shares the same neurotoxic mode of action as all other insecticides in this chemical class (Clark and Brooks 1989; WHO 1990). GCH is the active enantiomer of both LCH and CH, and as such would be expected to be up to twice as toxic to aquatic organisms as LCH and four times more toxic than CH (Wang et al. 2007). Furthermore, on the basis of information already known for other pyrethroids, it is reasonable to assume that invertebrates, in particular macroinvertebrate crustaceans and insects, will be highly sensitive to GCH, following exposure at environmentally relevant concentrations (Hill et al. 1994).

Laboratory ecotoxicity testing with GCH has confirmed that the aquatic crustacean *Daphnia magna* is highly sensitive, with a geometric mean 48 h EC_{50} of 66.9 ng/l (Marino and Rick 2000; Machado 2001), which is within the range reported for other pyrethroids (Brock et al. 2000). However, for other pyrethroids it has been reported that some insect and macrocrustacean species are significantly more sensitive than *Daphnia* (Maltby et al. 2005; Van Wijngaarden et al. 2005), and a priori it is reasonable to assume that this will also be the case for GCH. Therefore, the initial phase of the present investigation of GCH focused on determining the toxicity profile of GCH for a selection of freshwater invertebrates known to be sensitive to pyrethroids. Species sensitivity distribution (SSD) curves were generated for invertebrates typical of shallow freshwater ecosystems, to estimate the potential risks posed by GCH to natural ecosystems (Campbell et al. 1999; European Commission 2002; Brock et al. 2006). This was followed with an evaluation of the effects of GCH under

natural exposure conditions by conducting an outdoor multi-species model ecosystem test. The findings of the SSD approach and model ecosystem study for GCH were then compared to the outcome of comparable model ecosystem tests performed with other pyrethroids, including the racemic mixture LCH.

Material and methods

Toxicity tests

Static 96 h acute toxicity tests were performed with GCH, formulated as an emulsifiable concentrate (EC) containing 2.8% w/w GCH.

Test species and test conditions

Freshwater invertebrates were obtained from various local sources (Table 1) and acclimated to laboratory test conditions for at least 3 days prior to testing. During acclimation, organisms were provided with food and some form of shelter or substrate. A sub-sample of individuals of each species was taken to confirm identification, and only viable individuals were selected for testing. Organisms were maintained in a temperature-controlled room (20 ± 2°C) with a 14 h daylight cycle. During testing the exposure solutions were not aerated and test organisms were not fed. Test vessel type and exposure volumes for each species during testing are summarised in Table 1.

Test media and concentrations

The water used for testing originated from the water supply reservoir of the Sinderhoeve Experimental Station and had a hardness of 61 mg/l $CaCO_3$. The water was filtered over a 55 μm plankton net (Hydrobios, Kiel, Germany) before it was used for maintenance and testing of the organisms. The toxicity tests were performed with six concentrations of GCH, a control, and a formulation blank corresponding to that of the highest test solutions (Table 1).

Replication

Ten individuals were exposed per test vessel (replicate) with the exception of *N. maculata*, *C. punctata* and zygopterans where nine individuals were exposed in each vessel. For each treatment level three replicates were used

Table 1 Testing conditions for indigenous species in laboratory toxicity experiments performed with GCH

Taxon	Source[a]	Stage	Test vessel	Test volume/ individual (ml)	Test range[b] (ng GCH/l)
Insecta					
Diptera					
Chaoborus obscuripes	1	Larvae	Glass jar	100	0.11–36.1
Chironomini	2	Larvae	Glass jar	100	3.6–1,082
Ephemeroptera					
Cloeon dipterum	3	Nymph	Glass jar	100	3.6–1,082
Hemiptera					
Notonecta maculata	1	Adult	Segmented tank[c]	333	1.1–361
Corixa punctata	3	Adult	Segmented tank[c]	333	1.1–361
Zygoptera					
Coenagrionidae	1	Larvae	Segmented tank[c]	333	10.9–3,610
Crustacea					
Amphipoda					
Gammarus pulex	3	Adult	Glass jar[d]	100	1.1–361
Isopoda					
Asellus aquaticus	4	Adult	Glass jar[d]	100	1.1–361
Proasellus coxalis	5	Adult	Glass jar[d]	100	1.1–361

[a] *Source*: 1, microcosms, Sinderhoeve, Renkum, NL; 2, water supply basin, Sinderhoeve; 3, experimental ditch, Sinderhoeve; 4, ditch Veenkampen, Wageningen; 5, outlet waste water plant, Bennekom

[b] Test range: The range also included controls and formulation blanks (i.e. all formulation ingredients with the exception of the active substance GCH). For *C. obscuripes*: 0.00108 mg formulation blank/l; for *N. maculata–P. coxalis*: 0.0108 mg formulation blank/l; for chironomids–*C. dipterum*: 0.0324 mg formulation blank/l

[c] Test vessels divided into nine compartments to separate individuals

[d] Test vessels contained stainless steel gauze to act as a substrate

with the exception of *N. maculata*, *C. punctata* and Zygoptera, where two replicates were used.

Effect observations

After 24, 48 and 96 h the test organisms were visually evaluated by counting survivors. Effects were considered as lethal when no response of any kind was observed over a time period of 3–5 s after tactile stimulation. Observations for sublethal effects were also made, and all were considered a form of immobility. Scores of mortality were incorporated into those of immobility. At the 24 and 48 h observations, dead organisms were removed.

Chemical analysis

The application solutions were sampled to determine concentrations of GCH at the start ($t = 1$ h) and at the end of the toxicity tests ($t = 96$ h). GCH was extracted in hexane and concentrations measured by GLC (gas chromatograph: HP 5890) using ECD detection (detector: HP ECD; injector: HP Model: #G1513A; autosampler: HP 7673). Mean recoveries of GCH were between 99 and 106% for 10, 100 and 1,000 ng/l spiked samples.

Sensitivity calculations

EC_{50} and LC_{50} values and 95% confidence limits were calculated by a log concentration–logit effect regression method as described in Schroer et al. (2004). Within the regression, calculated L(E)C values were corrected for immobility/mortality in the controls. Tests were only considered valid if mean immobility/mortality in the control replicates did not exceed 20%. Toxicity parameters were based on nominal initial test concentrations. Results from replicate tests were combined into one regression analysis.

The SSD was defined as the cumulative frequency distribution of toxicity data. SSD analyses were conducted according to Aldenberg and Jaworska (2000) and the computer program ETX—version 1.403 (Van Vlaardingen and Traas 2002). Tests for log-normality were performed by means of Anderson-Darling goodness-of-fit test. Normality of toxicity data is assumed when p is ≥ 0.05 (Posthuma et al. 2002).

Microcosm study

Microcosms

The test was performed in 12 enclosures situated in one of the experimental ditches located at the Sinderhoeve Experimental Station, Renkum, The Netherlands (Drent and Kersting 1993). The enclosures consisted of polycarbonate, translucent cylinders (diameter: 1.05 m; height: 0.9 m), pushed c. 0.15 m into the sandy loam sediment [organic matter content of 5 cm top layer: 2.1 ± 0.01% (mean ± SD)]. Water depth was approx. 0.5 m and water volume c. 0.43 m^3. The enclosures were installed into the experimental ditch 17 days before treatment.

The microcosms simulated a mesotrophic, macrophyte-dominated freshwater system. The communities in the microcosms contained macroinvertebrates, zooplankton, phytoplankton and macrophytes. On the same day the enclosures were established, 100 *Gammarus pulex* and 60 *Asellidae* were introduced into each of the systems. These two taxa were introduced because they are known to be particularly sensitive to pyrethroids. Seven days before the first application, the above-sediment macrophyte biomass was 227 g dw/m^2 (mean). Dominant species were *Chara* sp., *Elodea* sp. and *Sagittaria* sp.

At the start, and throughout the study, concentrations of the total soluble nitrogen, ammonium, $NO_3 + NO_2$-nitrogen and orthophosphate generally were below detection limits (respectively, 2.2, 0.04, 0.02, and 0.03 mg/l). Total phosphate concentrations were at the limit of detection (0.02 mg/l).

Insecticide treatment

The study was conducted with a Capsule Suspension (CS) formulation containing 60 g/l GCH.

The treatment consisted of three applications of GCH, applied at 7 d intervals, to achieve initial concentrations of 0, 5, 10, 25, 50 and 100 ng GCH/l in the overlying water. All treatments, the controls inclusive, were in duplicate and were assigned randomly to the enclosures. The first treatment was on July 11, 2005. The applications were performed by pouring dosage solutions (1 l) over the water surface and the water was gently stirred to mix the compound throughout the water column. The control enclosures received water only.

GCH residues in water

Actual GCH concentrations were estimated by taking 500 ml depth-integrated samples (in duplicate) by means of a vacuum pump and stainless steel suction tubes. GCH was extracted with hexane, and analysed based on methodology developed by Cook and Olberding (2004) using GLC with ECD detection as previously described. The detection limit for GCH in enclosure water was 0.023 µg/l and mean recovery was 101.5 and 108.6% for the 10 and 100 ng/l treatments, respectively.

Table 2 Summary of endpoints investigated in microcosm study

Endpoint	Unit	Sampling weeks
Physico-chemical		
pH, temp., DO, EC	–, °C, mg/l, µS/cm	−2, …, 10
Macroinvertebrates		
Species composition	Numbers	−1, 0, 2, 4, 7, 10
Zooplankton		
Species composition	Numbers/l	−2, …, 3, 5, 7, 9, 10
Phytoplankton		
Chlorophyll-*a*	µg/l	−2, …, 3, 5, 7, 9, 10
Decomposition		
Particulate organic matter	g dw	−1, 0, 2, 4, 7, 10
Macrophytes		
Biomass	g dw/m^2	−1, 10

For a detailed description of methods for measuring the endpoints, see Van Wijngaarden et al. (2006). "…" indicates that sampling was weekly

Endpoints investigated

The endpoints measured are summarised in Table 2. Artificial substrates, consisting of litterbags and pebble baskets, were used to monitor the macroinvertebrate community; depth integrated water samples were taken to sample the zooplankton community; concentrations of chlorophyll-*a* were measured in water samples to provide an indicator of phytoplankton; decomposition of particulate organic matter (POM) was determined using leaf litter bags containing *Populus* × *canadensis* leaves which were placed at the sediment surface and left for periods of 2–3 weeks; above-sediment macrophyte biomass was determined shortly before, and at the end of the experiment.

Statistics

Prior to statistical analysis, macroinvertebrate and zooplankton data were Ln($ax + 1$) transformed, where x stands for the abundance value. For macroinvertebrates $a = 2$, and for zooplankton $a = 10$. This was done to down-weigh high abundance values and to approximate a normal distribution for the data (Van den Brink et al. 2000). NOEC calculations at taxon or parameter level ($p \leq 0.05$) were carried out using the Williams test (ANOVA; Williams 1972). The analyses were performed with the Community Analysis computer program (Hommen et al. 1994).

Effects on the macroinvertebrate and zooplankton communities were analysed by the principal response curves (PRC) method (Van den Brink and Ter Braak 1997, 1998, 1999). In addition to the overall significance of the effects of the treatment regime, each treatment was also compared to the controls to identify the NOEC at the community level. The NOEC calculations were carried out by applying the Williams test to the sample scores of the first principal component of each sampling date in turn (Van den Brink et al. 1996). Effects were considered consistent when they showed statistically significant deviations pointing in the same direction for at least two consecutive sampling points. The data were also evaluated for possible artefacts relating to small magnitude of measured counts, or having no treatment related concentration–response and/or no clear causality with community interactions or timing (European Commission 2002).

Results

Toxicity tests

Exposure concentrations

Mean (±SD) measured concentrations in the dose solutions were 87 ± 9% of the intended concentrations, while mean (±SD) measured concentrations after 1 h were 78 ± 13%. After 96 h, mean (±SD) concentrations in the test water reduced to 16 ± 6%.

Test conditions Minimum (±SD) dissolved oxygen levels and pH values during the tests were 7.8 ± 0.4 mg/l and 8.4 ± 0.7, respectively.

Sensitivity of species

Sensitivity of the tested species to GCH in terms of sublethal effects (EC_{50}) and mortality (LC_{50}) for all species is summarised in Table 3. In far most cases the lower and upper limits of the 95%-confidence interval were less than a factor of two relative to the median L(E)C_{50} values (Table 3). The zygopterans and the Chironomini (both insects) were relatively insensitive to GCH, while the most sensitive species were the insect *Chaoborus obscuripes* and the amphipod *G. pulex*. The calculated 48 and 96 h EC_{50} values for the species tested were used to perform sensitivity distribution (SSD) analyses (Fig. 1). Because Zygoptera consisted of a mixture of species (Table 3, footnote[d]), the EC values for this taxon were excluded from the analyses. The median fifth percentile hazard concentrations (HC_5) based on the 48 and 96 h EC_{50} values were 2.86 and 2.12 ng/l, respectively (Fig. 1).

Microcosm study

Exposure concentrations

The nominal initial concentration of GCH in the enclosures, based on the measured concentrations in stock

Table 3 Results of acute static laboratory toxicity tests with macroinvertebrates and GCH

Species	EC_{50} (95% confidence limits)			LC_{50} (95% confidence limits)		
	24 h	48 h	96 h	24 h	48 h	96 h
C. obscuripes	6.4[a]	6.4[a]	3.8 (3.6–4.0)	>36.1[b]	>36.1[b]	12.4 (9.3–16.4)
G. pulex	10.0[a]	5.0 (4.0–6.1)	9.2 (7.0–11.9)	38.4 (36.2–40.7)	16.1 (12.6–20.5)	10.3 (7.8–13.4)
N. maculata	13.0[a]	5.6[a]	4.6 (3.8–5.5)	>361[b]	65.7 (42.1–102)	15.2 (10.6–21.9)
C. punctata	13.3 (9.7–18.3)	12.3 (10.8–13.9)	12.3 (10.5–14.3)	>361[b]	64.6 (36.8–114)	21.3 (12.0–37.7)
P. coxalis	11.9[a]	17.7 (13.7–22.9)	16.6 (12.9–21.5)	>361[b]	218 (134–355)	74.6 (50.4–110)
A. aquaticus	12.6[a]	26.2 (17.0–40.4)	23.7 (16.9–33.4)	349 (167–727)	253 (123–519)	93.5 (57.8–151)
C. dipterum	12.1 (9.9–14.8)	24.8[a]	23.4 (17.1–31.9)	>1082[b]	887 (302–2,604)	56.3 (36.2–87.5)
Chironomini[c]	163 (125–213)	78.4 (58.1–106)	145 (106–198)	>1082[b]	>1082[b]	>1082[b]
Zygoptera[d]	173 (121–248)	304 (209–442)	322 (289–358)	>3610[b]	>3610[b]	1,004 (636–1,585)

Results are based on static tests with one application. L(E)C values are in ng GCH/l

[a] Confidence limits could not be calculated due to lack of partial responses or lack of clear dose-response relationship

[b] Value above highest tested concentration

[c] Chironomini were mainly *Microtendipes* gr. *chloris* with ca. 10% other species present

[d] Zygoptera were a mixture of at least four species (*Coenagrion puella/pulchellum*, *Enellagma cyathigerum*, *Ischnura* sp., *Coenagrion* sp.)

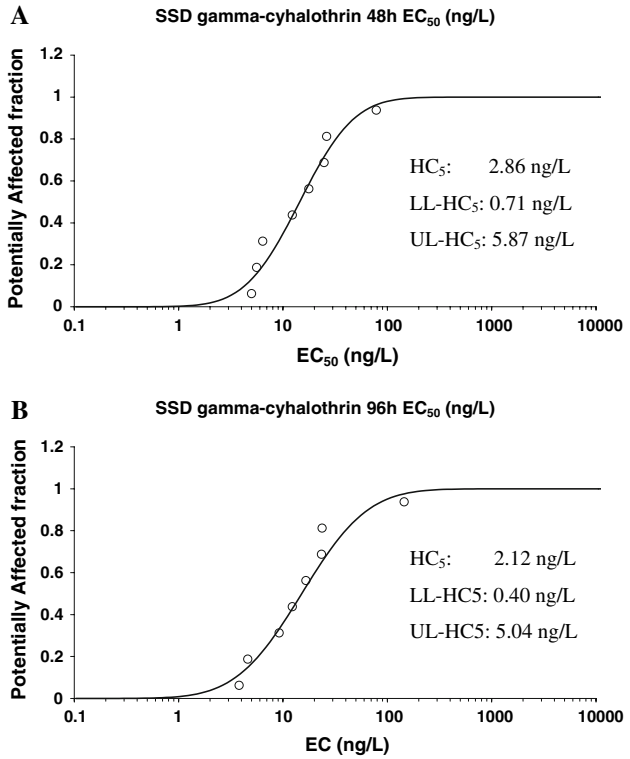

Fig. 1 Species sensitivity distribution (SSD) curves based on 48 h EC50 values (**a**) and 96 h EC50 values (**b**). HC5 is the median hazardous concentration for 5% of species calculated from the SSD. The LL-HC5 is lower limit of the 95% confidence interval of the median HC5. The UL-HC5 is the upper limit of the 95% confidence interval of the median HC5

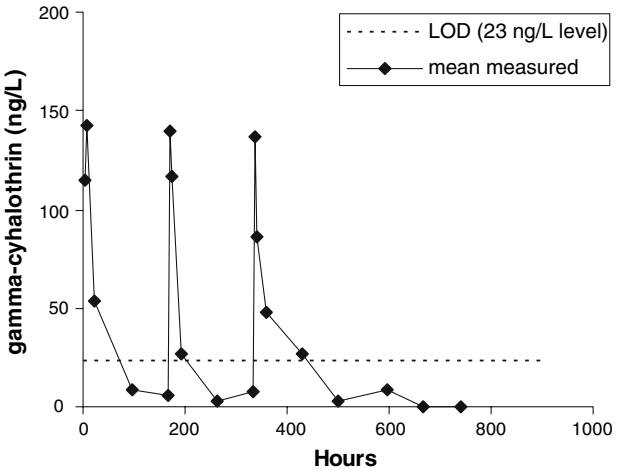

Fig. 2 Mean concentrations measured in the 100 ng GCH/l treatment of the two replicate enclosures. Measured values below 23 ng/l were <LOD, and are indicative only

solutions and assuming homogeneous mixing throughout the water column was on average 96.1% of intended initial concentration. The measured concentrations of GCH in the 100 ng/l treatment is illustrated in Fig. 2; the three applications of GCH were clearly visible as three concentration peaks in the water column followed by rapid dissipation between applications. The measured peak concentrations were between 113 and 152% of intended concentrations; this level of variation is commonly observed shortly after application of pyrethroids (e.g. Van Wijngaarden et al. 2006).

Macroinvertebrates

In total, 71 macroinvertebrate taxa were collected. Insects formed the majority of taxa, followed by molluscs. Besides

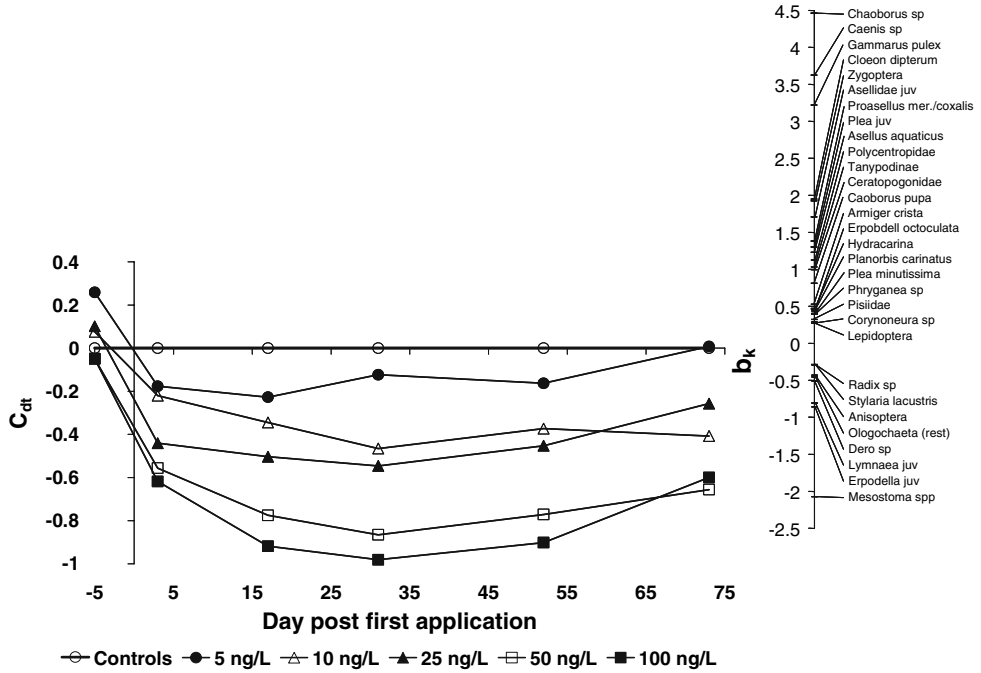

Fig. 3 Principal response curves (PRC) with species weight (b_k) for the macroinvertebrate data set, indicating the effects of GCH. Of all variance, 17% could be attributed to sampling date and is displayed on the horizontal axis. Differences between replicates accounted for 44% of all variance. Thirty-nine percent of all variance could be attributed to the treatment regime. Of this variance, 35% is displayed on the vertical axis. The vertical axis represents the differences in community structure between treatments and the controls expressed as regression coefficients (c_{dt}) of the PRC model. The species weight (b_k) can be interpreted as the affinity of the taxon to the PRC. Species with b_k between −0.25 and 0.25 are not shown in the diagram

the potentially sensitive group of insects, another sensitive group, the crustaceans, was also present. The PRC analysis indicated that 39% of all variance could be attributed to the treatment regime (Fig. 3). The macroinvertebrate community dynamics were significantly affected by exposure to GCH at concentrations above 5 ng/l (Table 4). The community response was dominated by a decrease in insects and crustaceans, with effects most pronounced at the 50 and 100 ng/l treatment levels. The taxa most affected were *Chaoborus* sp. (insect), *Caenis* sp. (insect) and *Gammarus pulex* (crustacean).

Table 4 Results of the Monte Carlo permutation test (p-value) and no-observed-effect concentrations (NOECs) on the community level (Williams test, $p \leq 0.05$) for the different treatment levels of gamma-cyhalothrin

Day	Macroinvertebrates		Zooplankton	
	p-value	NOEC	p-value	NOEC
−10		–	>0.05	≥100
−5/−3	>0.05	≥100	>0.05	≥100
2/3	0.002	50	>0.05	≥100
8		–	>0.05	≥100
15/17	0.005	50	>0.05	≥100
21		–	0.018	50
31/35	0.001	5	>0.05	≥100
49/52	0.004	25	>0.05	≥100
63		–	>0.05	≥100
70/73	>0.05	≥100	>0.05	≥100

–, no data. NOECs in ng a.i./l

Univariate analysis of populations indicated statistically significant deviations (Williams test, $p < 0.05$) on several consecutive sampling dates for 6 of the 71 taxa (Table 5). Of the taxa responding to GCH, *Chaoborus* sp., *Caenis* sp., *Gammarus pulex*, *Proassellus meridianus/coxalis* showed consistent treatment-related responses and the PRC indicated that *Chaoborus* sp. responded most explicitly (highest species weight (b_k) in Fig. 3). The $NOEC_{population}$ for *Chaoborus* sp. was identified as 5 ng/l (Table 5), while effects at higher concentrations were most severe after the third treatment at the 10 ng/l and higher treatment levels (Fig. 4a). Although the reduction was not statistically significant, at the 5 ng/l treatment level abundance numbers were about 25% of the controls on Day 17 (geometric mean abundance in controls: 40.1 vs 9.8 in the 5 ng/l treatment) and suggested a slight transient effect. Clear but partial reductions in *Chaoborus* abundance (ca. 50% of controls) were observed at the 10 ng/l treatment level, while pronounced long-term effects occurred at 25 ng/l and higher.

Table 5 NOECs (Williams test, $p < 0.05$) per sampling date for macroinvertebrate and zooplankton populations in enclosures (treatment levels, ng GCH/l) showing consistent deviations compared to controls in the post-treatment period

	Days after first application							
	2/3[a]	8	15/17[a]	21	31[a]/35	49/52[a]	63	70/73[a]
Macroinvertebrates								
Insecta								
Chaoborus sp.	10(↓)		10(↓)		10(↓)	5(↓)		5(↓)
Caenis sp.			10(↓)		<5(↓)	10(↓)		5(↓)
Ceratopogonidae	25(↓)		10(↓)					
Orthocadiinae			50(↓)		<5(↓)[b]	10(↑)		
Crustacea								
Gammarus pulex	50(↓)		25(↓)		25(↓)	25(↓)		
Proasellus mer./coxalis					25(↓)	50(↓)		
Zooplankton								
Cladocera								
Daphnia longispina	50(↓)	50(↓)	50(↓)	50(↓)				
Ceriophnia quadrangula		25(↓)	25(↓)					
Rotifera								
Cephalodella gibba			50(↑)	25(↑)	50(↑)	50(↑)		
Anuraeopsis fissa							5(↓)[c]	25(↓)[c]

Treatments were on Days 0, 7 and 14. Concentrations >NOEC showed significant increases (↑) or reductions (↓). The blank columns indicate no statistical significance at the highest treatment level, 100 ng/l

[a] Macroinvertebrate sampling

[b] Not considered treatment-related due to low and scattered abundance numbers, and a lack of any concentration–response relationship

[c] Late in study, causality with treatments unclear

Population dynamics indicate that recovery set in shortly after the third application.

For the *Caenis* sp. the $NOEC_{population}$ was determined to be below 5 ng/l (Table 5). The dynamics of *Caenis* sp. are summarised in Fig. 4b and were characterised by relatively high numbers on the last sampling dates in the controls, while interpretation of the data was difficult at the start of the experiment due to low abundance. Nevertheless, clear effects on *Caenis* sp., were apparent on at least two consecutive samplings from the 25 ng/l-treatment level and higher. At the 10 ng/l-treatment level, significant effects were observed on two isolated sampling days (Day 31 and 73). At the 5 ng/l-treatment level significantly lower numbers were only found on Day 31. A tendency of recovery was observed for the treatment levels of 25 ng/l and lower.

For *Gammarus pulex*, the $NOEC_{population}$ was 25 ng/l (Table 5) with clear and consistent effects observed at 50 and 100 ng/l (Fig. 4c). Statistical analysis indicated that recovery had occurred in the 50 and 100 ng/l treatment levels by the end of the study, although Fig. 4c illustrates that this was not the case.

Effects on *Proasellus meridianus/coxalis* were most severe after the third application and effects at the higher concentrations were only partial (Fig. 4d). Overall, the $NOEC_{population}$ was at the 25 ng/l treatment level (Table 5).

There were indications that Ceratopogonidae populations were reduced at treatment levels of 25 ng/l and higher (Table 5). However, this taxon was only present in low numbers in the first half of the study and so effects and recovery could not be properly evaluated. A statistically significant reduction in the Orthocladiinae was detected at all treatment levels on Day 31, while statistically significant increases were observed on Day 52 at treatment levels above 5 ng/l (Table 5). Validity of this statistical information, however, is weak since this species occurred in very low numbers and a concentration–response relationship was not clear as the species still occurred at the higher treatment levels of 10–100 ng/l.

GCH had no detectable effects on the non-arthropod macroinvertebrate taxa.

Zooplankton

A total 59 zooplankton taxa were identified in the enclosures during the experiment. Rotifers formed the majority of taxa, followed by crustaceans (Cladocera and Copepoda). The multivariate statistical analysis indicated

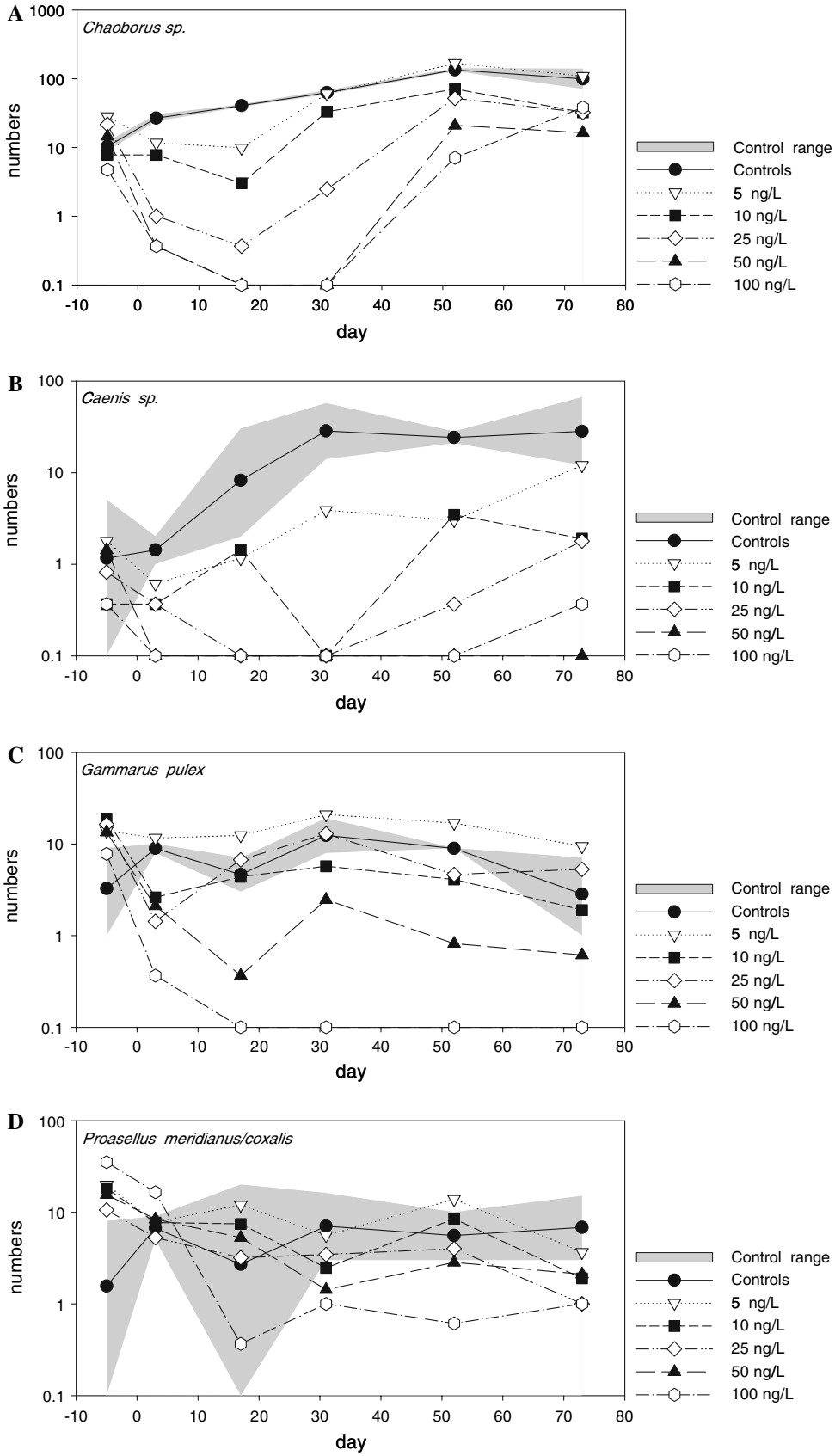

Fig. 4 Population dynamics, in numbers (geometric mean), of taxa showing consistent responses to GCH treatments. **a** *Chaoborus* sp., **b** *Caenis* sp., **c** *Gammarus pulex* and **d** *Proasellus meridianus/coxalis*. The value 0.1 denotes 0 numbers in the samples. Applications were on Days 0, 7 and 14

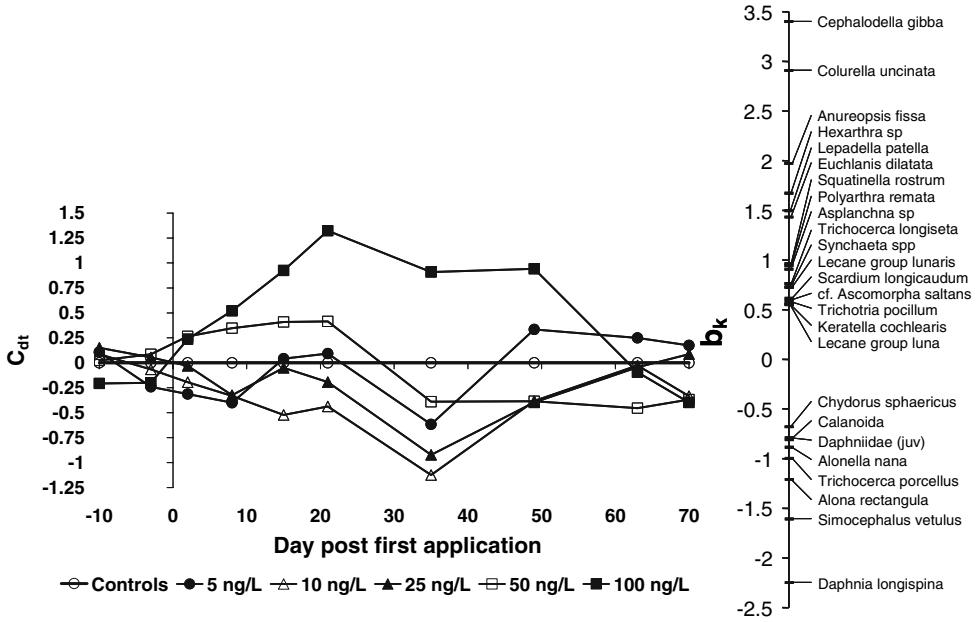

Fig. 5 Principal response curves (PRC) with species weight (b_k) for the zooplankton data set, indicating the effects of GCH. Of all variance, 32% could be attributed to sampling date and is displayed on the horizontal axis. Differences between replicates accounted for 38% of all variance. Thirty percent of all variance could be attributed to the treatment regime (which is a statistically non-significant part). Of this variance, a non-significant part of 19% is displayed on the vertical axis. The vertical axis represents the differences in community structure between treatments and the controls expressed as regression coefficients (c_{dt}) of the PRC model. The species weight (b_k) can be interpreted as the affinity of the taxon to the PRC. Species between b_k −0.5 and 0.5 are not shown in the diagram

that the zooplankton community was not affected significantly by the overall treatment regime of GCH (Fig. 5). The PRC analysis indicated a trend of deviation from the controls at the 100 ng/l treatment level. The zooplankton community response was characterised by a tendency of increases in rotifers and decreases of cladoceran populations. The populations correlating most to the diagram were the rotifers *Cephalodella gibba* and *Colurella uncinata* and the cladoceran *Daphnia longispina* as they have the highest species weights (b_k) in the PRC diagram. *C. gibba* and *C. uncinata* showed increased numbers. *D. longispina* showed reduced numbers (Fig. 6). The deviation of the highest treatment level was only significant on Day 21 and resulted in a $NOEC_{community}$ of 50 ng/l (Table 4).

Univariate analysis of populations indicated statistically significant deviations (Williams test, $p < 0.05$) on several consecutive sampling dates for 4 out of the 59 taxa encountered (Table 6). Consistent and statistically significant reductions in *D. longispina* populations only occurred at the 100 ng/l-treatment level (Table 5). A $NOEC_{population}$ of 25 ng/l was found for *Ceriodaphnia quadrangula* on two consecutive sampling dates, during the application period (Table 5). At the 100 ng/l-treatment level, the rotifer *C. gibba* showed consistent statistically significant increased abundance numbers (Table 5). The lowest NOEC (5 ng/l) was found for *Anuraeopsis fissa* on a single sampling date and was part of statistically significant reductions at the end of the experiment (Table 5). In the time period in which the three applications took place, no treatment-related responses were observed (Table 5). Because of a lack of causality with the treatments, the NOEC at 5 ng/l is not considered to be treatment-related.

The calanoids, cyclopoids and copepod nauplii did not show any consistent response at the treatment levels studied.

Chlorophyll

Phytoplankton chlorophyll-*a* concentrations did not show consistent treatment-related effects. The mean (±SD) phytoplankton chlorophyll-*a* concentrations during the entire experimental period including all enclosures was 34 ± 24 µg/l.

Decomposition

Generally, the reduction of POM was about 0.5 g dw. No consistent effects were observed.

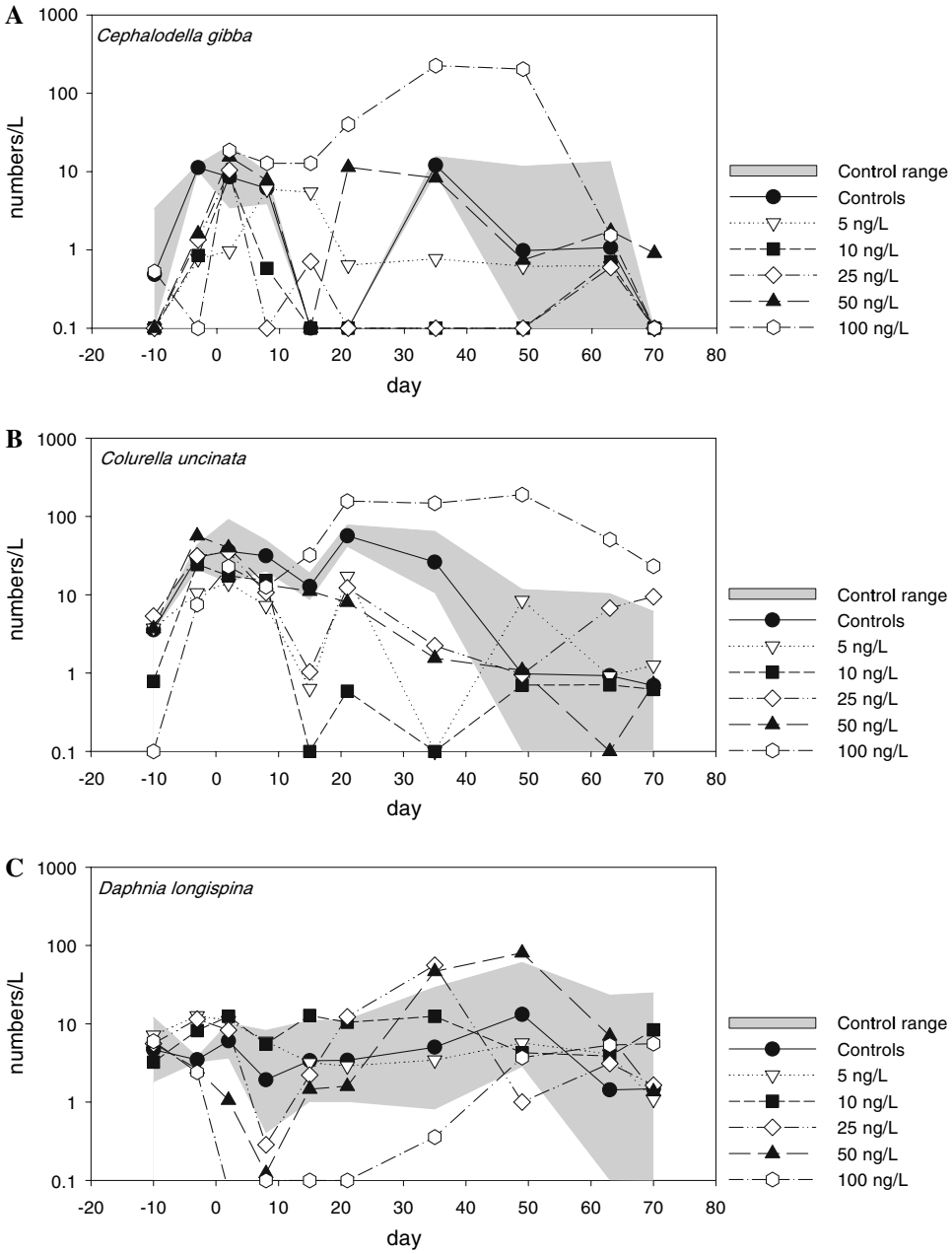

Fig. 6 Population dynamics, in numbers (geometric mean), of taxa showing consistent responses to GCH treatments. **a** *Cephalodella gibba*, **b** *Colurella uncinata* and **c** *Daphnia longispina*. The value 0.1 denotes 0 numbers in the samples. Applications were on Days 0, 7 and 14

Community metabolism

The GCH treatments did not result in pronounced impacts on community metabolism endpoints; mean oxygen levels were 9.0–10.1 mg/l and mean pH values were 8.1–8.8.

Discussion

Species sensitivity distribution

For GCH, the median HC_5 based on laboratory 48 h EC_{50} acute toxicity data for eight species from taxonomic groups known to be sensitive to pyrethroids was determined as 2.86 ng/l. The sensitivity distribution indicated *Chaoborus* sp. and *Gammarus pulex* to be the most sensitive of the tested indigenous species which corresponded with the effects observed in the microcosms, where these two were the most (*Chaoborus*) or one of the most sensitive species (*Gammarus*). When using the same taxa, agreement between short-term responses in static acute toxicity tests and model ecosystem studies has been reported on several occasions (e.g., Van Wijngaarden et al. 1996; Schroer et al. 2004). Our finding that the median HC_5 was protective towards the sensitive taxonomic groups, also in case of repeated applications, in micro/mesocosms is in line

Table 6 Summary of effects observed in enclosures treated with GCH

Endpoint	Treatment (ng GCH/l)				
	5	10	25	50	100
PRC macroinvertebrates	1	2	2	3	3
Macrocrustaceans	1	1–2↓[a]	1–2↓[a]	5↓	5↓
Insecta (excl. *Chaoborus* sp.)	2↓[b]	2↓[b]	5↓	5↓	5↓
Chaoborus sp.	1–2↓[c]	3↓[d]	5↓[d]	5↓[d]	5↓[d]
Non-arthropod macroinvert	1	1	1	1	1
PRC zooplankton	1	1	1	1	2
Cladocera	1	1	1	3↓[e]	3↓[f]
Rotifera	1	1	1	2↑[g]	3↑[h]
Copepoda	1	1	1	1	1
Chlorophyll-*a*	1	1	1	1	2
Community metabolism	1	1	1	1	1

Explanation of effect classes: the numbers in the table follow the effect classes as described by Brock et al. (2000) and summarised in SANCO/3268/2001 rev.4 (final), 2002. 1, Effect could not be determined; 2, Slight effect; 3, Pronounced short-term effect; 4, Pronounced effect in a short-term study (not relevant for this study); 5, Pronounced long-term effect; ↓, decrease of endpoint; ↑, increase of endpoint. PRC: principle response curves of macroinvertebrate or zooplankton community. Within each endpoint category the most sensitive measurement endpoint is presented

[a] *G. pulex,* partial reduction directly after first application, though not statistically significant

[b] *Caenis* sp., transient reduction on Day 31

[c] *Chaoborus*, partial reduction on Day 17 after third application, though not statistically significant

[d] *Chaoborus*, recovery clearly evident, but numbers remained lower than controls

[e] *C. quadrangula,* statistically significant reduction on Day 8 and Day 15

[f] *D. longispina* and *C. quadrangula*, decreased numbers

[g] *C. gibba*, transient increase on Day 21

[h] *C. gibba*, increase from Day 15 to 49

with other studies that compared the SSD approach to responses in aquatic model ecosystems using insecticides (e.g., Schroer et al. 2004; Maltby et al. 2005).

Sensitive groups and $NOEC_{community}$

Effects observed in the microcosm study may be summarised into effect classes as illustrated in Table 6. In this study the $NOEC_{population}$ for the most sensitive species was at, or close to 5 ng/l with reductions in abundance at 5 ng/l only detectable on a single sampling date. The transient population effects at this concentration had no detectable impact on the overall invertebrate community, and so the lowest test concentration was determined to be the $NOEC_{community}$. At increasingly higher concentrations GCH induced more severe effects on the sensitive insect taxa, with pronounced long-term effects and lack of full recovery within the duration of the study evident at 25 ng/l and above. The macroinvertebrates *Gammarus pulex* and *Asellus aquaticus* were less sensitive than *Chaoborus* sp. and *Caenis* sp. but also demonstrated clear long-term effects and lack of full recovery at the two highest concentrations. Zooplankton were less sensitive than the macroinvertebrates, and short-term effects on some cladocerans and a rotifer only occurred at the 50 and 100 ng/l-treatment levels. A slight transient effect on the zooplankton community was only detected at 100 ng/l. No treatment-related effects were observed on non-arthropod macroinvertebrates and copepods.

Community interactions

GCH caused direct negative effects on sensitive macroinvertebrates, with profound short-term effects on the macroinvertebrate community (specifically some sensitive insects and macro crustaceans) detected at the 50 and 100 ng/l-treatment levels. At these treatment levels, the most sensitive zooplankton species also showed a response, with few cladoceran species declining in abundance while some rotifers increased in abundance.

The release of predation pressure caused by the reduction of *Chaoborus* sp.—which is very sensitive to GCH and an important predator of cladocera—did not result in significantly higher population densities of cladocerans at the treatment levels below 100 ng/l. The decrease in cladoceran densities (*D. longispina*, *C. quadrangula*) at the 100 ng/l-treatment level may have reduced food competition and mechanical filtering, causing an increase of rotifer populations (*C. gibba*). The treatment did not lead to pronounced indirect effects in the form of increases in the algae.

The treatments did not appear to affect community metabolism. The reduction in sensitive macroinvertebrate shredders (e.g. *Gammarus pulex* and *Proasellus coxalis/meridianus*) did not lead to considerable reductions in decomposition of POM.

GCH and other model ecosystem studies with pyrethroids

The effects of GCH, the active enantiomer of the synthetic pyrethroid LCH, were consistent with the effects observed in model ecosystem studies performed with this latter compound, but at lower concentrations (Schroer et al. 2004; Maltby et al. 2005; Brock et al. 2006). Studies with LCH also found that insects, in particular *Chaoborus* sp., were the most sensitive populations for both phytoplankton and macrophyte dominated communities, with clear effects occurring at treatment levels of 10 ng/l and above, and

pronounced effects (Classes 3–5) on sensitive Baetidae, Caenidae, Asellidae and Gammaridae at treatment levels of 16–25 ng/l and higher (Farmer et al. 1995; Roessink et al. 2005; Van Wijngaarden et al. 2006). Thus, it appears that the aquatic risk profile of LCH, in terms of relative species sensitivity, population effects and community response in a complex exposure system can largely be attributed to the single active enantiomer GCH. This indicates that the fate and behavior of the active enantiomer in LCH is not dissimilar to that of the inactive enantiomer, as indicated by the rapid dissipation of GCH from the water column in our study (with 40% of dose remaining in the water column after 1 day) which was similar to that reported for LCH (Leistra et al. 2003; Roessink et al. 2005). Therefore, the microcosm data illustrate that the two enantiomers making up LCH have similar fate profiles, with the single enantiomer GCH demonstrating up to twice the level of toxicity to aquatic invertebrates as the racemate LCH. Therefore, the concerns associated with potential enantioselectivity when assessing the aquatic risk of pyrethroids, as raised by Ali et al. (2003) and Liu et al. (2005), are not manifested in the case of LCH when compared to the fate and effects of its active enantiomer.

Besides the information for the cyhalothrins, a considerable amount of data is available from model ecosystem studies with other synthetic pyrethroids performed under various experimental conditions (see review Van Wijngaarden et al. 2005). To place the GCH model ecosystem data into context with these other studies we expressed exposure concentrations as Toxic Units (TU) (Van Wijngaarden et al. 2005) and corresponding observed effects were assigned to one of the effect classes (Table 6). To be in line with the other pyrethroids, where TU was based on the most sensitive standard species (either *Daphnia* or a fish), TU for GCH was based on the geometric mean 96 h LC_{50} for *Lepomis macrochirus* (47.2 ng/l; Marino and Rick 2001a, b). When focusing on the sensitive endpoint categories it is clear that the concentration–response relationship does not deviate from that of the other pyrethroids, though GCH tended towards the less sensitive side (Fig. 7). The use of either *Daphnia* or fish for setting the TU for the individual pyrethroids has little impact on the resulting distribution of the effect responses since *Daphnia* and fish differ little in sensitivity (about a factor of 1.5 (mean), range: 1.08–2.6), with in approximately half of the cases fish being more sensitive (Brock et al. 2000).

Overall, for the various pyrethroid studies, effects start to become apparent in the most sensitive categories 'Microcrustaceans', 'Macrocrustaceans' and 'Insects' from about 0.01 TU (Fig. 7a–c). In the range 0.01–0.1 TU they relate especially to slight effects (Class 2). At higher exposure concentrations (>0.1 TU), clear effects (Classes 3–5) are regularly reported for 'Microcrustaceans', 'Macrocrustaceans' and 'Insects' (Fig. 7a–c).

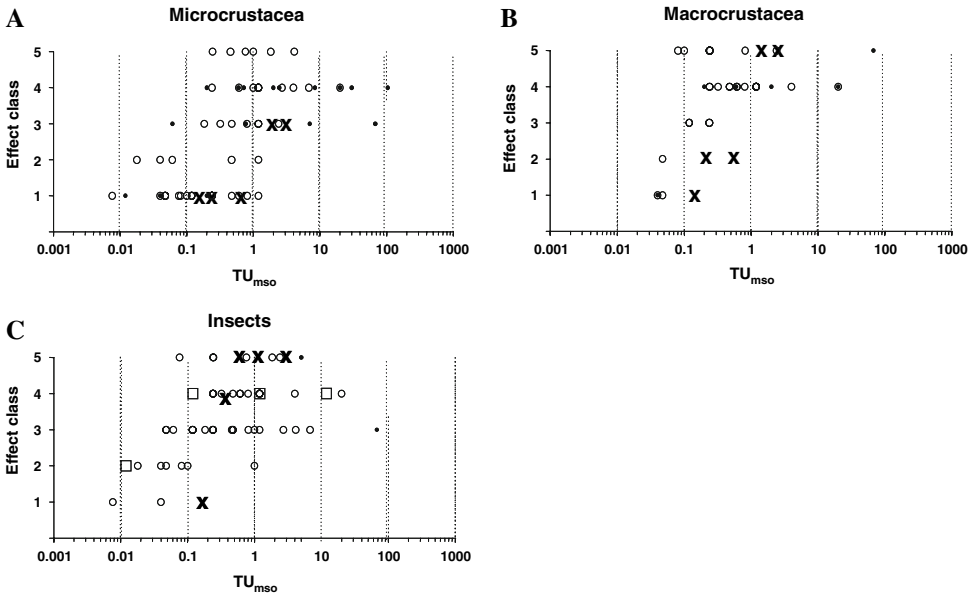

Fig. 7 Effects of insecticides with synthetic pyrethroids in model ecosystem studies (after Van Wijngaarden et al. 2005). Reported concentrations were transformed into toxic units scaled to the most sensitive standard test organism (TU_{mso}). Effects for the potentially sensitive endpoint categories Microcrustacea (**a**), Macrocrustacea (**b**) and Insects (**c**) are given. The effects were summarised into Effect classes: 1, no significant effect, 2, slight effect, 3, clear short-term effect (<8 weeks), 4, clear effect in short-term study (recovery moment unknown), 5, clear long-term effect (>8 weeks). *Closed circles* (●) indicate experiments with a single application. *Open circles* (○) and *squares* (□) indicate experiments with multiple applications or chronic exposure, respectively. The responses of the present GCH study (multiple applications) are indicated with X

In conclusion it is apparent from the results of a range of model ecosystem experiments with non-persistent insecticides that, within a single compound, threshold levels for effects are very similar when they contain representatives of sensitive taxonomic groups (in this case arthropods) and when exposure patterns are similar (Brock et al. 2006). Consequently there is considerable confidence when extrapolating threshold level effects (Classes 1–2) observed in good quality model ecosystem studies to different spatio-temporal situations in the case of pyrethroids.

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