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Direct and Indirect Effects of the Fungicide Carbendazim in Tropical Freshwater Microcosms

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Abstract Direct and indirect effects of the fungicide carbendazim on ecosystem structure and functioning were studied ≤8 weeks after application (nominal concentrations: 0, 3.3, 33, 100, and 1000 μg/L) to outdoor microcosms in Thailand. Direct effects on macroinvertebrates are discussed in detail in a separate article. The present article presents the effects on other end points and discusses the hypothesized ecologic effect chain. Negative treatment effects on the zooplankton community were only recorded for the highest carbendazim treatment ($NOEC_{community}$ = 100 μg/L). The rotifer *Keratella tropica*, cladocerans (*Moina micrura*, *Ceriodaphnia cornuta*, and *Diaphanosoma* sp.), and cyclopoid copepods were decreased or even eliminated at this treatment level. The decrease in zooplankton and macroinvertebrate abundances was accompanied by an increase in numbers of several tolerant invertebrates, presumably caused by a release from competition and predation. The death of sensitive invertebrates probably also led to an overall decreased grazing pressure because increased levels of chlorophyll-*a* and bloom of the floating macrophyte *Wolffia* sp. were noted. The increase in primary producers is discussed to be the probable cause of changes in physicochemical water conditions, eventually resulting in an anoxic water layer during the last 3 weeks of the experiment. This is likely to have resulted in decreased invertebrate abundances noted in that period. Furthermore, the decreased decomposition of *Musa* (banana) leaves observed 8 weeks after application is considered to be the indirect effect of a decreased microbial activity resulting from these anoxic water conditions, rather than a direct toxic effect of carbendazim.

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As a result of a shift from traditional subsistence farming toward intensive-crop farming, pesticide use in Thailand has increased considerably since the Green Revolution in the late 1960s (Pingali 1997; Thapintha and Hudak 2000; Satapornvanit et al. 2004). Furthermore, decreasing prices for rice and a production restructuring program by the Thai Ministry of Agriculture in 1994 led to a conversion of land used to cultivate rice, cassava, coffee, and pepper into more pesticide intensive crops of fruits and vegetables (Jungbluth 2000). Because fungicides are mainly used for the cultivation of fruit and vegetables, fungicide imports doubled between 1991 (2,087 tons) and 1996 (4,446 tons), after which fungicide imports increased gradually to 6,732 tons in 2003 (Jungbluth 2000; Chunyanuwat 2005).

Like in many other tropical countries, few studies into the fate and environmental side effects of pesticides have been conducted in Thailand (Bourdeau et al. 1989;

Ecobichon 2001; Racke 2003; Kwok et al. 2007). The Thai ecotoxicologic literature consists almost entirely of determinations of LC_{50} values for various freshwater species using laboratory tests (Campbell and Parnrong 2001). Hence, fate and effects on ecosystem level have barely been studied.

Model ecosystems have often been used as surrogates for ecosystems in pesticide risk evaluations. Because of the variable and complex test conditions in the field, where often multiple stressors (e.g., different pesticides and/or eutrophication) are present at the same time, results of field studies are difficult to interpret. In contrast, laboratory toxicity tests have little ecologic realism and do not take into account certain aspects, such as indirect effects (e.g., interactions between trophic levels) and recovery potential (Brock et al. 2004). Microcosms and mesocosms link true experimental reproducibility with ecologic realism and have therefore been considered a bridge between the laboratory and the field (Brock et al. 2000).

This article presents the direct and indirect effects of the fungicide carbendazim on the zooplankton community, chlorophyll-*a* of phytoplankton and periphyton, decomposition, and physicochemical parameters noted in a microcosm study carried out in tropical Thailand. A separate article deals with the fate of carbendazim in the microcosms and discusses treatment effects on the macroinvertebrate community in detail (Daam et al. 2009). The overall hypothesized ecologic effect chain is discussed in the "Discussion" section.

Materials and Methods

Microcosms and Application of the Test Substance

The experiment was conducted using 12 rectangular microcosms (length and width 1 m and height 1.15) containing a sediment layer of 10 cm and 1000 L water. The test systems were set up outdoors at the Asian Institute of Technology (AIT) near Bangkok (Thailand). After a pretreatment period of 6 weeks, carbendazim was applied as Bavistin FL (BASF AG, Ludwigshafen, Germany) in nominal concentrations of 0 ($n = 4$), 3.3 ($n = 2$), 33 ($n = 2$), 100 ($n = 2$), and 1000 ($n = 2$) µg/L. Because only a few model ecosystem studies evaluating fungicides have been carried out in temperate countries, carbendazim was chosen because reference studies evaluating single-peak (Slijkerman et al. 2004) and chronic (Cuppen et al. 2000; Van den Brink et al. 2000) exposure were available for this compound, enabling a comparison of effects in the present study with those reported under temperate semi-field conditions. Details on the experimental design and carbendazim analysis are described in Daam et al. (2009).

Decomposition

Litter bags filled with *Musa* (banana) leaves were used to study decomposition of particulate organic matter. *Musa* leaves were collected from banana trees on the AIT campus, which are not treated with pesticides. Preparation of *Musa* leaves was done according to the method described for *Populus* leaves by Cuppen et al. (2000). To this end, collected *Musa* leaves were cut, leached three times for 2 days to remove the more easily soluble humic compounds, and dried for 72 h at 60°C. Subsequently, subsamples were dried at 105°C to establish the 60°C/150°C dry weight ratio. The nylon litter bags (mesh 0.2 mm) contained 2 g dry weight (60°) *Musa* leaves and were closed with stainless steel wire. One litter bag was retrieved from each microcosm each after an incubation period of 2, 4, and 8 weeks.

Water Sampling

A 10-L water sample was collected in a bucket on a weekly basis by taking several depth-integrated subsamples using a Perspex tube. One liter was used for phytoplankton chlorophyll-*a* and alkalinity analysis. The bucket was then partially emptied into the microcosm from which it had been taken, leaving 5 L in the bucket. This remainder was passed through a zooplankton net (mesh size 60 µm) and preserved with formalin (final concentration 4% V/V) to examine treatment effects on the zooplankton community. In addition, at 2-week intervals, a 1-L water sample was taken approximately 10 cm below the water surface in plastic bottles for nutrient analysis.

Zooplankton

Subsamples of the zooplankton sample were counted with an inverted microscope (magnification × 100 to 400). Rotifers and cladocerans were identified to the lowest taxonomic level possible. Copepods were divided into nauplii (immature stages), calanoids, and cyclopoids (mature stages), whereas Ostracoda were not further identified. Numbers were recalculated to numbers per liter microcosm water.

Chlorophyll-*a* Phytoplankton and Periphyton

Phytoplanktonic chlorophyll-*a* measurements were made with a known volume of the 1-L water sample taken as previously described. A known volume was concentrated over a Whatman GF/C glass fibre filter (mesh size 1.2 µm) until the filter was saturated. Filters were then air dried and extracted the same day using the method developed by Moed and Hallegraeff (1987).

Water-Quality End Points

Dissolved oxygen (DO), pH, electrical conductivity (EC), and temperature were measured approximately 10 cm below the water surface 2 weeks before application and on a weekly basis after application. On sampling days, measurements were made in the morning (just after sunrise) as well as at the end of the afternoon (just before sunset). DO and pH measurements were made using a YSI (Yellow Springs, OH, USA) model 58 oxygen meter connected to a YSI 5739 probe and a Consort (Turnhout, Belgium) C523 pH meter, respectively. EC and temperature were measured with a Consort C532 conductivity meter. Alkalinity levels were determined at weekly intervals in 100-mL subsamples from the 1-L water sample obtained as previously described by titrating with 0.05 N HCl until a pH of 4.2 was reached. The concentrations of ammonia, nitrate, and ortho-phosphate were analyzed at 2-week intervals according to the methods described by the American Public Health Association (1992).

Statistical Analysis

No observed effect concentration (NOEC) calculations were made with the Williams test using Community Analysis, version 4.3.05 (Hommen et al. 1994), and statistical significance was accepted at $p < 0.05$. Before analysis, zooplankton data were $\mathrm{Ln}(x + 1)$ transformed, where x stands for the abundance value. Effects on zooplankton community level were analyzed by principal response curves (PRCs) using the CANOCO software package, version 4.5 (Ter Braak and Smilauer 2002). The significance of the PRC diagram was tested with the Monte Carlo permutation test, and the $\mathrm{NOEC_{community}}$ was calculated for each individual sampling date by applying the Williams test to the sample scores of the first principal component of each sampling date. For a detailed explanation and rationale, please refer to Daam et al. (2009).

Results

Zooplankton

In terms of overall abundance, the control zooplankton community was dominated by rotifers, copepods, and cladocerans, followed by ostracods. Rotifera were the most diverse group with 11 species, 4 of which belonged to the *Brachionus* family, whereas Cladocera were represented by 4 taxa. Immature stages of copepods (nauplii) had a high abundance throughout the experimental period in the controls, with an average of 380/l. In these microcosms, cyclopoid copepods increased with time, leading to slightly higher numbers compared with calanoid copepods at the end of the experiment.

The PRC of the zooplankton data shows a clear deviation from controls at the highest treatment level (Fig. 1), which is in agreement with results from the permutation tests and $\mathrm{NOEC_{community}}$ calculations (NOEC = 100 µg/L; Table 1). The dynamics of the four most distinctive taxa in the PRC are shown in Fig. 2a–d. The rotifer *K. tropica*

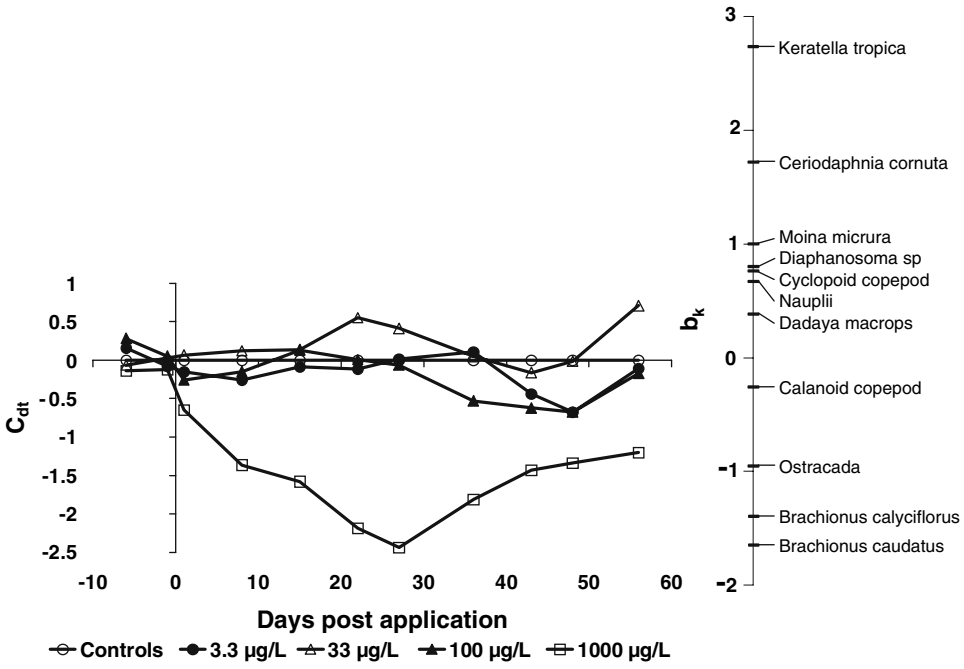

Fig. 1 PRCs resulting from the analysis of the zooplankton data set indicating the treatment effects of carbendazim on the zooplankton community. Of all variance, 21% could be attributed to sampling date; this is displayed on the horizontal axis. Forty-two percent of all variance could be attributed to treatment level. Of this variance, 40% is displayed on the vertical axis. The lines represent the course of the treatment levels in time. The species weight (b_k) can be interpreted as the affinity of the taxon with the PRCs. A Monte Carlo permutation test indicated that a significant part of the variance explained by treatment level is displayed in the diagram ($p = 0.024$)

Table 1 Results of Monte Carlo permutation (p) and Williams tests on PCA coordinates ($NOEC_{community}$; µg/L) performed for each sampling date for the zooplankton data set

Day	p	NOEC
−6	0.797	>1000
−1	0.905	>1000
1	0.124	>1000
8	0.065	100
15	0.251	>1000
22	0.087	>1000
27	0.031	100
36	0.003	100
43	0.01	33
48	0.028	100
56	0.004	100

(Fig. 2a) and the cladoceran *C. cornuta* (Fig. 2b) have the highest species weight in the diagram and are thus indicated to have decreased because they were exposed to the highest carbendazim concentration. Two other rotifers, *Brachionus caudatus* (Fig. 2c) and *B. calyciflorus* (Fig. 2d) have the lowest species weight, pointing to increased numbers at this treatment level. These effects were confirmed by analysis at the species level, which also indicated increased abundances of other rotifers (*Lecane closterocerca* and *Euchlanis* sp.) and decreased abundances of other cladocerans (*M. micrura* and *Diaphanosoma* sp.; Table 2). The univariate analysis also indicated treatment effects on copepods and ostracods (Table 2). Cyclopoid copepods had lower numbers in the highest dosed microcosms 1 day after application and in the last 3 weeks of the experiment (Fig. 2e; Table 2). Abundances of Calanoida (Fig. 2f) and immature stages of copepods (nauplii; Fig. 2g) were also decreased by the end of the experiment, although Calanoida were increased in the fourth week after application (Table 2). These decreased abundances of copepods in weeks 6 through 8 were accompanied by increased numbers of ostracods in weeks 6 and 7 in the two highest carbendazim treatments (Fig. 2h; Table 2).

Phytoplanktonic and Periphytonic Chlorophyll-*a* Concentrations

Chlorophyll-*a* content of the phytoplankton showed a significant increase 5 weeks after application in the microcosms treated with the highest carbendazim dose, whereas the same systems had decreased chlorophyll-*a* values 8 weeks after application (Table 3). Periphytonic chlorophyll-*a* levels were significantly increased at this treatment level in the samples taken 4 weeks after application (Table 3).

Water-Quality Parameters

Several effects on water-quality parameters were noted, particularly in the last 3 weeks of the experiment (Table 3). Carbendazim led to significantly lower DO concentrations and production, as well as pH, temperature, and nitrate levels, especially at the highest carbendazim concentration. At this treatment level, DO concentrations decreased <5 mg/L from 6 weeks after application onward (data not shown), and this was accompanied by an increase in alkalinity levels (Table 3).

Decomposition

Figure 3 shows the percentage of *Musa* (banana) leaf decomposition in the litter bags. The decomposition of the leaves in the control test systems after decay periods of 2, 4, and 8 weeks were 36%, 50%, and 87%, respectively (Fig. 3). The microcosms treated with 1000 µg carbendazim/L had a significantly lower decomposition after an 8-week incubation (66%; Fig. 3).

Bloom of the Floating Plant Wolffia sp.

Five weeks after application, the floating plant *Wolffia* sp. started to emerge in the microcosms treated with the highest carbendazim concentration. Within 1 week, this resulted in a complete coverage of the water surface by this macrophyte. In the other microcosms, *Wolffia* sp. only covered a small part (<10%; data not shown) of the water surface and only by the end of the experiment (i.e., 8 weeks after application).

Discussion

Sensitivity of the Zooplankton Community

In microcosm studies evaluating single-peak and chronic (4 weeks) treatments of carbendazim carried out in the temperate zone, NOECs calculated for the zooplankton community were 3 and 33 µg/L, respectively (Van den Brink et al. 2000; Slijkerman et al. 2004). In these studies, cladocerans were identified as the most susceptible zooplankton taxa, and effects on individual cladoceran taxa were reported at concentrations of 30 µg/L (Slijkerman et al. 2004) and 33 µg/L (Van den Brink et al. 2000). In the present study, however, consistent treatment effects on the zooplankton community and decreased abundances of cladocerans and their taxa richness were only noted at a concentration of 1000 µg/L (NOEC = 100 µg/L; Tables 1, 2). The lower sensitivity of the zooplankton community in the present study was probably the result of the limited number

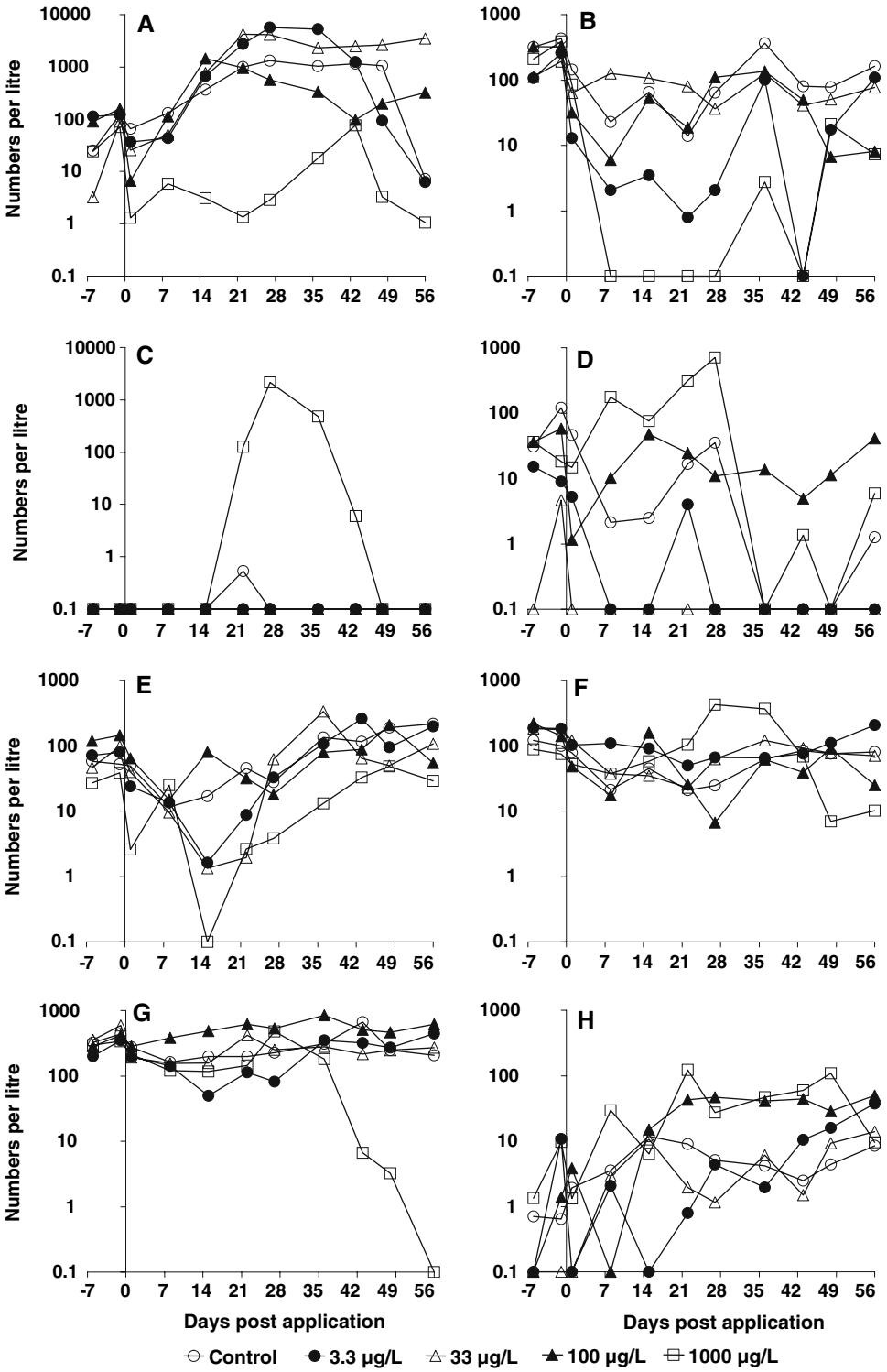

Fig. 2 Dynamics of the zooplankton taxa that were most important in the PRC analysis as well as dynamics of copepods and ostracods. Figure **a**–**d** show the geometric means per liter of the two taxa with the highest positive [*K. tropica* (**a**) and *C. cornuta* (**b**)] and highest negative weights [*B. caudatus* (**c**) and *B. calyciflorus* (**d**)] in the PRC. Figure **e**–**h** show the geometric means per liter of cyclopoid copepods (**e**), calanoid copepods (**f**), nauplii (**g**), and ostracods (**h**). A value of 0.1 denotes absence of the taxon

of cladoceran species and their relatively low abundances, whereas rotifers dominated the zooplankton communities. Tropical freshwater zooplankton communities, including those in Southeast Asia (Segers 2001), have indeed been reported to be largely dominated by rotifers (Kutikova 2002). Lower and higher diversity and abundance of, respectively, cladocerans and rotifers imply that the chance of encountering sensitive representatives is also higher and lower, respectively.

Most consistent treatment effects were noted for the rotifer *K. tropica*, and this taxon was indicated to decrease in abundance at 100 µg/L 1 day after application (Fig. 2a; Table 2). Remarkably, the temperate species *K. quadrata* was among the most affected zooplankton taxa in the study

Table 2 NOECs per sampling week for zooplankton taxa that showed a significant response in the Williams test calculations ($p < 0.05$)

	Sampling week											
	−1	0	0.1	1	2	3	4	5	6	7	8	Figure
Rotifera	>	>	>	>	>	>	>	>	100↓	100↓	>	
K. tropica	>	>	33↓	100↓	100↓	100↓	100↓	100↓	>	100↓	>	2a
B. caudatus	>	>	>	>	>	100↑	100↑	100↑	100↑	>	>	2c
L. closterocerca	>	>	>	>	100↑	>	>	>	>	>	>	
Lepadella patella	100↑	>	>	>	>	>	>	>	>	>	>	
Euchlanis sp.	>	>	>	100↑	>	>	>	>	>	>	>	
Cladocera	>	>	>	100↓	100↓	100↓	100↓	>	>	>	100↓	
M. micrura	>	>	>	100↓	>	100↓	>	>	33↑	>	100↓	
Diaphanosoma sp.	>	>	>	>	>	>	>	100↓	100↓	>	3.3↓	
C. cornuta	>	>	>	>	100↓	>	100↓	100↓	100↓	>	100↓	2b
Copepoda	>	>	>	>	>	>	100↑	33↑	100↓	100↓	100↓	
Cyclopoid	>	>	100↓	>	>	>	>	>	100↓	100↓	33↓	2e
Calanoid	>	>	>	>	>	>	100↑	>	>	>	33↓	2d
Nauplii	>	>	>	>	>	>	>	>	100↓	100↓	100↓	2g
Ostracoda	>	>	>	>	>	>	>	>	33↑	33↑	>	2h

Concentrations (in μg/L) showed significant increases (↑) or decreases (↓); > indicates a NOEC of >1000 μg/L

Table 3 NOECs (in μg/L) calculated for water-quality end points and chlorophyll-*a* concentrations of phytoplankton and periphyton

	Sampling week										
	–2	–1	0	1	2	3	4	5	6	7	8
DO	>	>	>	>	>	>	>	>	100↓	100↓	100↓
pH	>	>	>	>	>	>	>	>	100↓	100↓	100↓
EC	>	>	>	>	>	100↓	>	100↓	100↓	>	>
Temperature	>	>	>	>	>	>	33↓	>	100↓	100↓	100↓
Alkalinity	>	>	>	>	>	>	>	>	100↑	>	100↑
Nitrate	>	>	>	>	>	>	>	>	100↓	>	>
Chlorophyll-*a*											
Phytoplankton	NM	>	NM	>	>	>	>	100↑	>	>	100↓
Periphyton	NM	>	NM	NM	>	NM	>	100↑	>	NM	>

Significant treatment effects (Williams test, $p < 0.05$) resulted in either increased (↑) or decreased (↓) values in affected microcosms

"> " NOEC > 1000 μg/L; *NM* not measured

by Slijkerman et al. (2004) and was one of the most sensitive rotifers in the study by Van den Brink et al. (2000). In a microcosm study evaluating the fungicide triphenyltin acetate, *K. quadrata* was also the most sensitive zooplankton taxon and showed a quick negative response to the fungicide (Roessink et al. 2006). Another *Keratella* species, *K. coclearis*, was the most sensitive taxon of six rotifer taxa tested to the fungicide pentachlorophenol as determined by swimming behavior and vulnerability to predation (Preston et al. 1999). Apparently, the genus *Keratella* includes the most susceptible representatives of rotifers and often even the zooplankton community to fungicides. Other rotifer taxa (*B. caudatus*, *B. calyciflorus*, *L. closterocerca*, *Euchlanis* sp.) were indicated to increase in abundance (Figs. 1, 2; Table 2). Similarly, increased abundances of tolerant rotifer taxa were also reported in Slijkerman et al. (2004) and van den Brink et al. (2000).

Abundances of cyclopoid copepods decreased 1 day after application in the highest carbendazim concentration and returned to control levels on day 7 (Fig. 2e; Table 2). However, they were eliminated on day 14 at this treatment level, although no significant difference could be demonstrated due to variation in controls and lower treatments, where after they steadily increased in numbers (Fig. 2e). Van den Brink et al. (2000) also found decreased abundances of cyclopoid copepods, but this lasted only 4 weeks

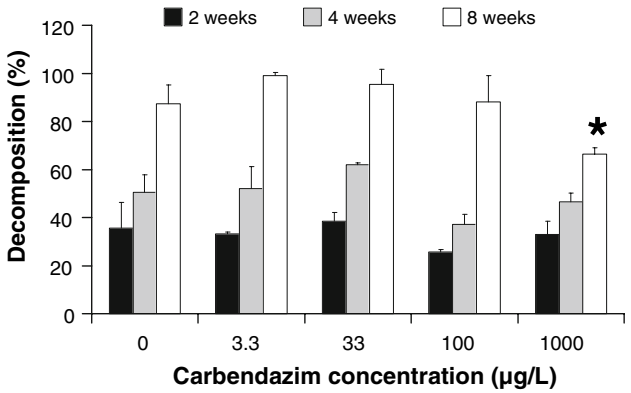

Fig. 3 Decomposition (%) of *Musa* leaves after an incubation period of 2, 4, and 8 weeks for the different treatments. Significant deviations from controls (Williams test; $p < 0.05$) are indicated with an *asterisk*

after the start of the treatment. These investigators discussed that this phenomenon was probably the result of a decrease in immature copepod stages, i.e., nauplii, rather than a direct toxic effect of carbendazim on mature copepods (Van den Brink et al. 2000). In the present study, however, the effect was immediate, and no decrease in nauplii was noted (Fig. 2g; Table 2), indicating a direct treatment-related effect on mature cyclopoids. The decrease in abundances of the rotifer *K. tropica* may also have played a role because most cyclopoid copepods are micropredators feeding on small invertebrates, whereas calanoid copepods are mostly planktonic fine-particle filter feeders (Alekseev 2002). In line with this, the rotifer community on day 1 and week 2 consisted almost entirely (97% and 95%, respectively) of the large rotifer *B. calyciflorus*, which may have been too large for the cyclopoid copepods to handle. In addition, *B. calyciflorus* has been reported to increase body size and produce longer spines in response to substances produced by copepods (Kutikova 2002). One and 3 weeks after application, substantial contributions to the rotifer community of the smaller rotifers *Euchlaris* sp. (48%) and *B. caudatus* (45%) may explain the increasing trend in abundances of cyclopoid copepods on those sampling days (Fig. 2e).

Effects on Other End Points and Ecologic Effect Chain

The hypothesized ecologic effect chain is summarized in Fig. 4. Carbendazim led to decreased abundances and even an elimination of various macroinvertebrates (Corixidae, Baetidae, Oligochaeta, Mollusca, Ampullaridae, and Ancylidae; Table 4) and zooplankton taxa (Cladocerans, cyclopoid copepods, and *K. tropica*). As discussed previously, the decreased abundances of cyclopoid copepods most likely resulted from decreased numbers of *K. tropica*. The decreased competition for food as a result of the death of sensitive invertebrates probably allowed tolerant invertebrates to increase in numbers (Fig. 4). Decreased predation pressure is also likely to have contributed to this phenomenon because water boatmen (Corixidae) have been reported to primarily feed on small invertebrates (Dudgeon 1999). The increase in tolerant invertebrates may have compensated partly the decreased grazing pressure caused by the death of sensitive invertebrates because significant increases in periphytonic and phytoplanktonic chlorophyll-*a* content were only calculated 5 weeks after application (Table 3).

A bloom of the floating plant *Wolffia* sp. was observed in the microcosms treated with 1000 µg carbendazim/L at the end of the experiment. Van den Brink et al. (2000) also found an increase in macrophytes (*Elodea nuttallii*) after carbendazim applications of 330 and 1000 µg/L and explained this by a decreased presence of pathogens of these macrophytes, directly or indirectly caused by carbendazim. Although this may have played a role in the present study, the *Wolffia* outbreak is more likely to have resulted from the complete elimination of apple snails (Ampullariidae; Table 4). Apple snails are known to be efficient grazers of aquatic macrophytes and have even been reported to leave the water column to forage for plants (Carlsson et al. 2004; Dudgeon 1999). The complete coverage of the water surface by *Wolffia* sp. may partly explain the decrease in several water-quality parameters (DO, pH, EC, and temperature) as well as algal biomass, as indicated by the decreased chlorophyll-*a* content of the phytoplankton (Fig. 4; Table 3). The reduction in algal biomass may have worsened the effects on the physicochemical parameters by way of decreased primary production and the decomposition of the phytoplankton biomass. At the same time, the primary production by *Wolffia* probably led to a decrease in nitrate levels, but apparently this did not compensate for the decreased DO levels. Presumably, a large part of the gas exchange by the dense *Wolffia* mat occurred with the ambient air, rather than with the water column. In line with this, Morris and Barker (1977) indicated that much of the oxygen produced by *Wolffia* mats is lost to the atmosphere.

During the last 2 weeks of the experiment, anoxic water conditions (morning 0.4 ± 0.1 mg L^{-1}; afternoon 1.5 ± 1.3 mg L^{-1}) and a complete absence of phytoplanktonic chlorophyll-*a* were recorded for the microcosms exposed to 1000 µg carbendazim/L. This probably resulted in decreased abundances of several invertebrates and/or hampered recovery of populations affected by the carbendazim treatment. For example, an increasing trend of *K. tropica* between 3 and 6 weeks after application was followed with a decreasing trend in weeks 7 and 8 in the microcosms exposed to the highest application (Fig. 2a). Remarkably, ostracods abundances increased in zooplankton samples taken from these tanks during that period (Fig. 2h; Table 2).

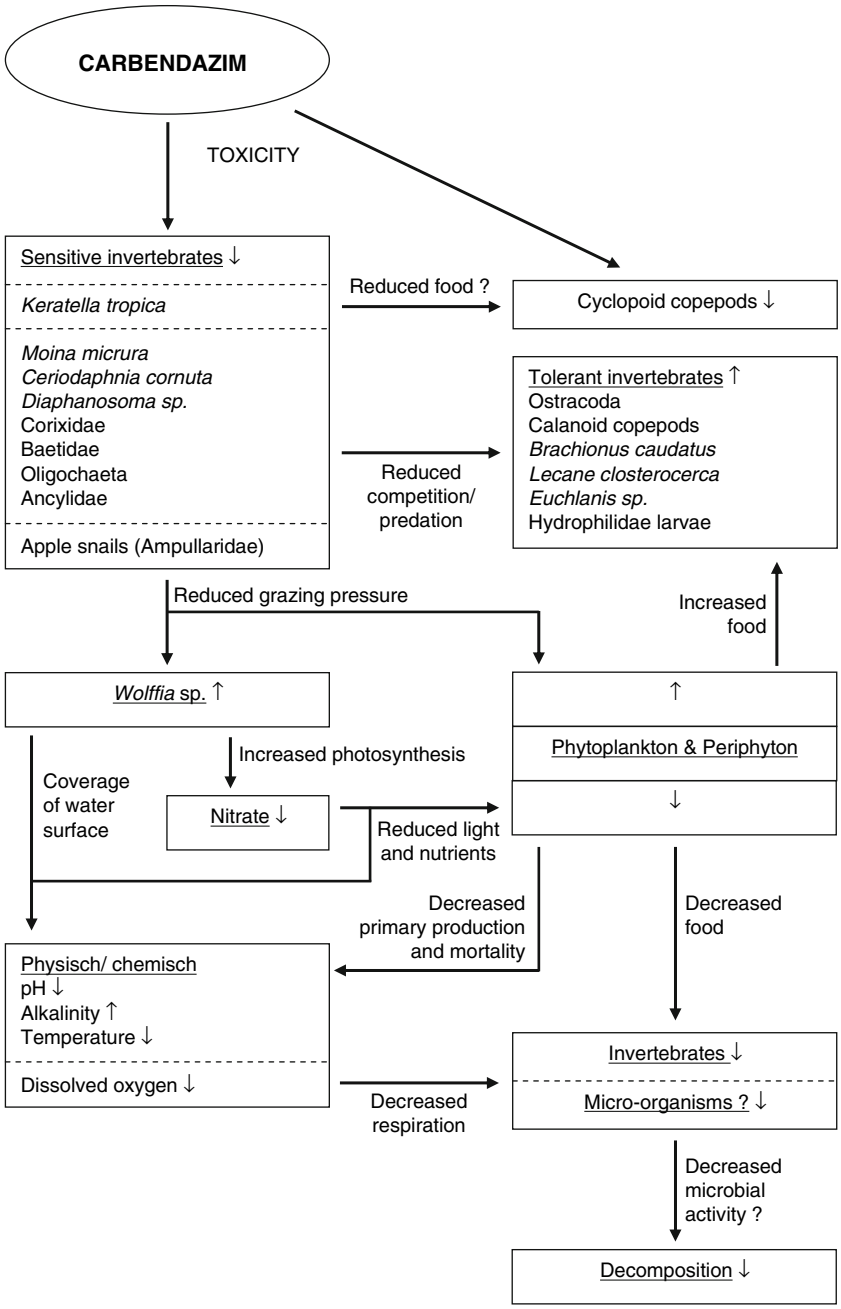

Fig. 4 Schematic overview of the hypothesized ecologic effect chain after application of 1000 µg carbendazim/L. Arrows indicate increase (↑) or decrease (↓) relative to controls

Ostracods have indeed been reported to be resilient and indicative of stressed environments where most zooplankton is eliminated, such as anoxic water conditions (Victor 2002; Corbari et al. 2004).

Decomposition of the *Musa* leaves was decreased in the highest treatment tanks after a decay period of 8 weeks (Fig. 3). It is unlikely that this was a direct effect of carbendazim because no effect was observed after a 4-week decay period, when most of the fungicide had already disappeared. A more plausible explanation is a decrease in microbial activity as a consequence of the anoxic water conditions. Because microorganisms were not studied in the present experiment, we can not confirm this hypothesis.

In conclusion, direct toxic effects of carbendazim were most pronounced on macroinvertebrates; only the lowest concentration tested did not exert effects (NOEC = 3.3 µg/L; Table 4). Although NOECs of 33 µg/L were calculated on individual sampling dates, consistent effects on the zooplankton community and populations occurred only at a concentration of 1000 µg/L. Therefore, the sole use of standard test organisms (mostly *Daphnia*, fish, and algae) for the risk assessment of fungicides may be questionable

Table 4 NOECs per sampling week calculated for the macroinvertebrate community (multivariate analysis) as well as macroinvertebrates that showed a significant response in the Williams test calculations (univariate analysis; $p < 0.05$)

	Sampling day				
	−2	13	25	45	55
Macroinvertebrate community	>	3.3	3.3	33	3.3
Arthropoda; Insecta					
Corixidae	>	3.3↓	3.3↓	3.3↓	3.3↓
Baetidae	>	100↓	33↓	>	>
Hydrophilidae larvae	>	>	33↑	>	>
Annelida					
Oligochaeta	>	100↓	100↓	33↓	100↓
Mollusca					
Ampullariidae	>	100↓	>	>	>
Ancylidae	>	33↓	100↓	>	>

Concentrations (μg/L) showed significant increases (↑) or decreases (↓)

Source: Daam et al. (2009)

"> " NOEC > 1000 μg/L

because the most susceptible taxa (macroinvertebrates) are not represented (Cuppen et al. 2000; Daam et al. 2009). In Europe, the only macroinvertebrate that is required to be tested (and only in certain cases [e.g., for insect growth regulators]) for first-tier risk assessment of pesticides is the freshwater midge *Chironomus riparius*. However, most sensitive macroinvertebrates to carbendazim in temperate regions were indicated to be "worm-like" taxa (e.g., Oligochaeta, Turbellaria, Hirudinae; Cuppen et al. 2000), whereas water boatmen (Corixidae) were the most sensitive macroinvertebrate representatives in the present study (Table 4). In laboratory toxicity tests by Domingues et al. (2009), the tropical chironomid *Kiefferulus calligaster* appeared only moderately sensitive to carbendazim. Effects on the cholinesterase activity were only demonstrated at concentrations of 5000 and 1700 μg/L after exposure to carbendazim for 3 and 6 days, respectively (Domingues et al. 2009). Hence, the inclusion of sensitive macroinvertebrate representatives in the risk assessment of fungicides in both temperate and tropical areas should be considered (Cuppen et al. 2000; Daam et al. 2009). Several indirect effects of carbendazim on the end points measured could be indicated with the experimental set-up applied. These effects appeared to be confined to the 1000 μg/L treatment, and the hypothesized chain of events occurring after carbendazim application was therefore elucidated for this treatment level (Fig. 4).

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