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Effects of Exposure Duration of Herbicides on Natural Stream Periphyton Communities and Recovery

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Abstract. To improve risk estimates for herbicides in streams, the sensitivity of natural periphyton communities to four herbicides (metribuzin, hexazinone, isoproturon, and pendimethalin) was examined in experiments including varying exposure duration and a recovery phase. Effect parameters included assimilation of ^{14}C and concentration of diagnostic pigments as proxies for photosynthetic activity and algal group composition, respectively. The results indicated that isoproturon, metribuzin, and hexazinone affected the photosynthetic activity of periphyton at distinctly lower concentrations than the effect concentrations published for standard single-species growth-tests with phytoplankton species. Pendimethalin did not show effects on the photosynthetic activity of periphyton at the concentrations tested. The effect concentration (EC_{50}) of isoproturon and metribuzin decreased by one to two orders of magnitude when the duration of exposure increased from 1 h to 24 h, while hexazinone had a stimulating effect on the photosynthetic activity of periphyton after 1 h exposure and inhibiting effect after 24 h exposure. The photosynthetic activity after exposure to metribuzin for 1, 2, 6, 18, 23, or 48 h recovered almost completely after 48 h in herbicide-free water. However, different periphyton groups responded differently to metribuzin exposure: Chlorophytes were severely affected by exposure and did not recover, whereas diatoms and especially cyanobacteria recovered well. Overall the study showed that the effects of herbicides on periphyton are strongly affected by the duration of exposure, and even short-term exposure may have distinct effects on the periphyton community.

During the past decade it has been documented that pesticides frequently occur in measurable concentrations in Danish streams (*e.g.,* County of Funen 1999; County of Aarhus 1999; NERI 2002). A significant transport of pesticides from agricultural areas to the streams by drift, and runoff has also been documented throughout Europe and North America. The highest concentrations occurring during the spraying season and during periods of high precipitation (Kreuger 1998; Baker and Richards 1989; Lundbergh *et al.* 1995; County of Funen 1989; Liess and Schultz 1999). The frequency and concentration of pesticides in stream water can be variable. In Danish streams pesticides occurred in 7–60% of the samples analyzed, and the concentrations ranged between 0.001–3 $\mu g\ L^{-1}$ (NERI 2002). Correspondingly, in an agricultural area in southern Sweden during the period 1990–1996, selected herbicides were found in 73% of the stream samples with concentrations of up to 45 $\mu g\ L^{-1}$ (Krueger 1998).

Although the effects of insecticides on macroinvertebrates in the streams have been documented repeatedly (*e.g.,* Pusey *et al.* 1994; Liess and Schultz 1999; Sibley *et al.* 1991), the effects of herbicides on the autotrophic organisms in streams are less obvious and therefore more difficult to establish. In small streams periphyton and macrophytes are the most important autotrophic organisms; still, standard risk assessments today include test with single-species of phytoplankton and to a lesser extent duckweed (*e.g.,* Lewis 1995). In comparison to the extensive literature on herbicide effects on phytoplankton studies on periphyton are much fewer (*e.g.,* Goldsburg and Robinson 1986; Herman and Kaushik 1986; Gurney and Robinson 1989; Jüttner *et al.* 1995; Detenbeck *et al.* 1996; Pérès *et al.* 1996; Van den Brink *et al.* 1997), and very few (if any) have examined the responses to short-term exposures that are likely to occur during runoff events. The application of single-species tests for risk assessment has been repeatedly criticized because of the great variability in sensitivity of freshwater species and the associated uncertainty related to the selection of the most sensitive species to test (*e.g.,* Cairns 1986; Fletcher 1989; Swanson *et al.* 1991). Though the documentation is meager a similar variation among species or groups is likely to occur within periphyton (Kosinski 1984; Herman and Kaushik 1986; Detenbeck *et al.* 1996). Besides, in single-species tests the only possible recovery is restricted to adaptation within a species (Okay and Gaines 1996), and community changes are precluded. Like phytoplankton, periphyton has short generation times and might be able to recover quickly at the functional level.

In contrast to phytoplankton, periphyton is attached to surfaces in a polysaccharide matrix along with heterotrophic microbes, fungi, and detritus, which may serve as an effective barrier preventing the transfer of a contaminant (Wang and

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Douglas 1999). Hence, to arrive at realistic risks for periphyton it is important to include such complex matrix in experiments.

With the aim to improve risk evaluated for herbicides in streams this study had three objectives: (1) to determine the sensitivity of natural periphyton communities, (2) to determine the influence of exposure duration on periphyton communities to herbicides, and (3) to determine the potential for recovery.

The investigated herbicides were hexazinone, metribuzin, isoproturon, and pendimethalin. The herbicides are all currently used and are potentially toxic to algae. Hexazinone (3-cyclohexyl-6-dimethylamino-1-mmethyl-1,3,5-triazine-2,4[1*H*, 3*H*]-dione) and metribuzin (4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazine-5-one) are both triazine herbicides that may inhibit transport of electrons in photosystem II and the chloroplast (Hassall 1990). Isoproturon (3-[4-isopropylphenyl]-1,1-dimethylurea) is a urea herbicide that has the same mode of action as triazines. Pendimethalin (*N*-[1-ethylpropyl]-2,6-dinitro-3,4-xylidine) is a dinitroaniline herbicide, and the major effects are on the growth of roots. Half-live for the four herbicides vary between 2–3 months (Tomlin 1994). Solubility in water is very different between the four herbicides (metribuzin 1,000 mg/L, hexazinone 33,000 mg/L, isoproturon 6.5 mg/L, and pendimethalin 0.275 mg/L). Hexazinone is used as a postemergence contact herbicide effective against annual, biennial, and perennial weeds. Isoproturon is used in pre-and postemergence control of annual grasses and annual broad-leaved weeds. Metribuzin and pendimethalin is used in pre-and postemergence control of many grasses and broadleaved weeds.

Functional effects of the herbicides were quantified by the photosynthetic activity and structural effects as community composition by diagnostic pigments. For one herbicide (metribuzin), recovery was studied by transferring the periphyton community to herbicide-free water.

Materials and Methods

Collecting Periphyton Communities

Three frames, each containing 170 round glass discs with a diameter of 1 cm, were deployed in the mesothrophic and uncontaminated (due to almost no agricultural activity in the catchment area) stream Esrum Mølleå, located in the northern part of Sealand, Denmark, in September 1999 and May 2000. After 2–3 weeks the glass discs were visibly colored by colonizing periphyton growing on the glass surfaces, and the discs were transported to the laboratory nearby. The discs were gently transferred to a white tray containing stream water and sorted with regard to the density of attached periphyton. Only glass discs, which were uniformly covered with periphyton, were used in the experiments. Each disc selected was transferred to a glass vial containing 10 ml filtered stream water, to which 100 μM $NaNO_3$, 16 μM Na_2HPO_4, and 50 μM $Na_2SiO_3 \cdot 5H_2O$ was added to ensure that nutrients did not limit the growth of the periphyton in the experiments.

Exposure to Herbicides

In September 1999, the glass discs with periphyton were exposed to pendimethalin, isoproturon (two experiments), metribuzin, or hexazinone for 24 h at concentrations of 0, 0.4, 2, 10, and 50 μg L^{-1}. Additional concentrations of 250 and 1,250 μg L^{-1} for isoproturon and metribuzin were included in the experiments. Furthermore, for studying the effects of short-term exposure to pesticides, periphyton was also exposed to hexazinone, isoproturon, and pendimethalin in the concentrations 0, 0.4, 2, 10, and 50 μg L^{-1} for 1 h. The glass vials were incubated at *in situ* temperature (16°C) and light intensity (250 μE $m^{-2}s^{-1}$) in an incubator in the laboratory. The effect of the pesticides was determined by measuring the photosynthetic activity by adding 1 μCi ^{14}C-bicarbonate (International Agency for ^{14}C Determination, Denmark) to the vials (five replicates) by the end of the exposure period and incubating the vials for 1 h as described. Adding acetic acid until the pH reached 2 stopped the incubations. The water was evaporated by drying the samples at 60°C. The release of incorporated ^{14}C was enhanced by the addition of 1 ml concentrated dimethyl sulfoxide. After 30 min, 10 ml scintillation cocktail (Ultima-Gold, Packard) was added. The activity, as disintegration per minute (dpm), was calculated from the counts per minutes (cpm) data using an external standard technique and appropriate correction factors. The abiotic fixation in all experiments $<0.5\%$ was estimated as the fixation of ^{14}C in samples killed by the addition of formaline to a final concentration of 1%.

Effects of Exposure Duration

In May 2000 the effect of duration of exposure (1, 2, 6, 18, 23, 48 h) was studied at different concentrations (0, 0.4, 2, 10, and 50 μg L^{-1}) of metribuzin. Two hundred seventy vials, each containing one glass disc with a natural community of periphyton, were prepared for the experiment. The discs were incubated with metribuzin at the different concentrations, and after 1, 2, 6, 12, 24, and 48 h the photosynthetic activity was determined in triplicate as described. In addition the effect of metribuzin on the periphyton community was evaluated by analysis of pigment composition. Three glass discs with periphyton from the same concentration were wrapped in a GF/F filter, immediately frozen in liquid nitrogen, and analyzed for pigment within 2 months by HPLC as described.

Recovery Experiment

After exposure of 1, 2, 6, 12, 24, and 48 h to metribuzin, the water was decanted from each vial and immediately replaced by uncontaminated filtered stream water with nutrients added. After 48 h in clean water, the photosynthetic activity was determined in triplicate as described above. The corresponding recovery of the pigment composition of the periphyton communities exposed for 2, 24, or 48 h were investigated after 48 h in herbicide-free water.

Calculation of Effect Concentrations

No effect concentrations (NEC) and effect concentrations (EC_{50}) were calculated for the photosynthetic activity measurements using log-linear interpolation as described in Petersen and Gustavson (1998). In some instances NEC could not be determined. Instead, the lowest observed effect concentration (LOEC) was determined.

Pigment Analyses

For HPLC analyses of the pigment composition the filter package with the three glass discs was thawed and placed in 6 ml 90% acetone, sonicated on ice for 10 min, and extracted for 24 h at 4°C. The filter and cell debris were filtered from the extract using disposable syringes

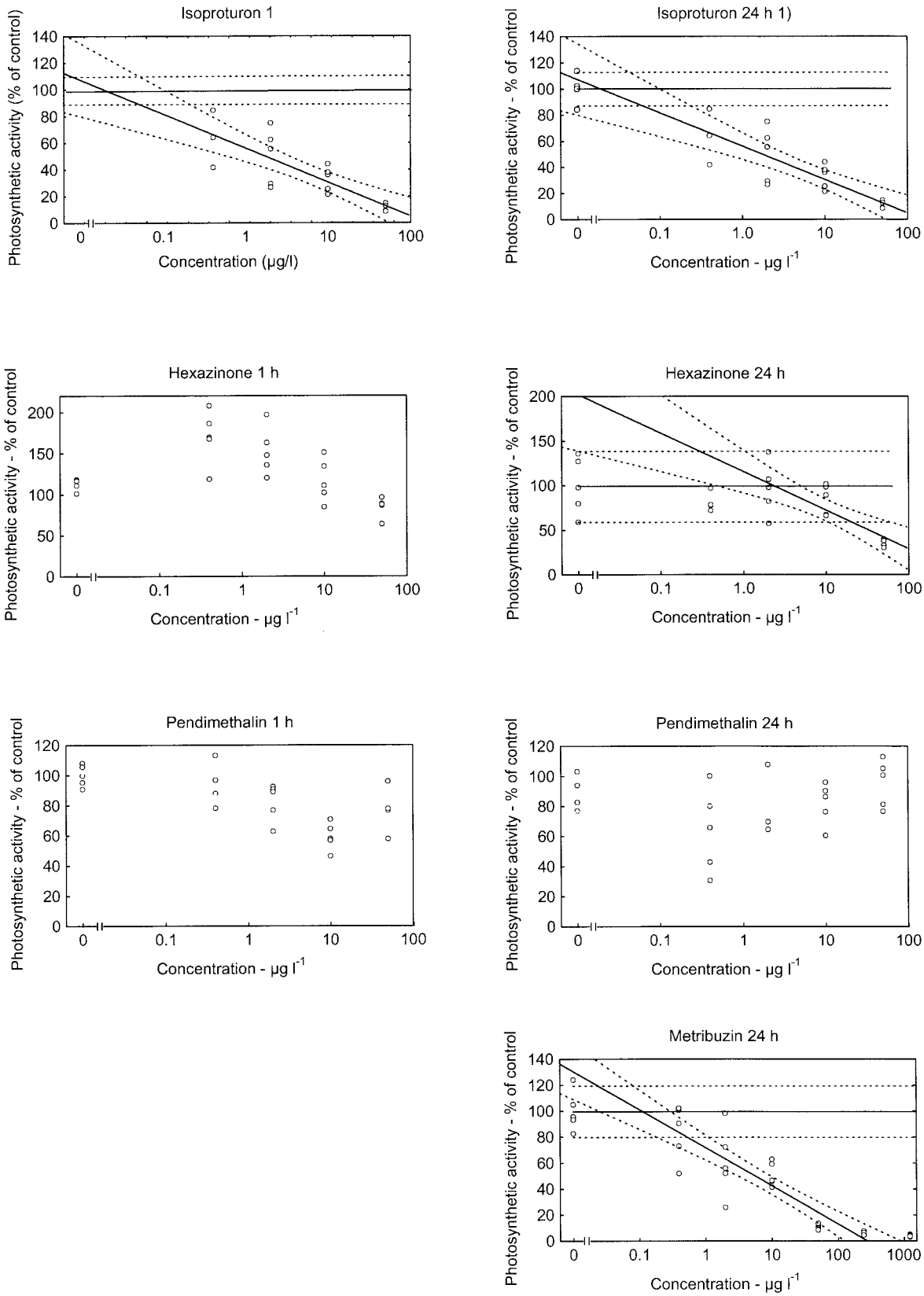

Fig. 1. Effect of four different herbicides on the photosynthetic activity of periphyton communities. Effect was measured after 1 and 24 h exposure. Dotted lines indicate 95% confidence limits of the controls and the linear regression

Table 1. No effect concentrations (NEC) and effect concentrations (EC_{50}) for the herbicides investigated during 1 and 24 h of exposure

	1-h Exposure		24-h Exposure	
Chemical	NEC ($\mu g\ L^{-1}$)	EC_{50} ($\mu g\ L^{-1}$)	NEC ($\mu g\ L^{-1}$)	EC_{50} ($\mu g\ L^{-1}$)
Isoproturon	1.00	11.28	0.019	1.74
Metribuzin	Missing	Missing	0.11	5.57
Hexazinone	Both stimulating and inhibiting	Both stimulating and inhibiting	2.29	32.88
Pendimethalin	No effects	No effects	No effects	No effects

Table 2. No effect concentrations (NEC) and effect concentrations (EC_{50}) for metribuzin as duration of exposure

Hours of Exposure	NEC ($\mu g\ L^{-1}$)	EC_{50} ($\mu g\ L^{-1}$)
1	Inconsistent effect*	34.00
2	0.6	9.57
6	Inconsistent effect*	33.16
18	Inconsistent effect*	23.73
23	2.35	15.23
48	1.37	31.56

* Both stimulating and inhibiting effect.

and 0.2-μm Teflon syringe filters. One milliliter of extract and 0.3 ml water was transferred to HPLC vials, and the vials were placed in the cooling rack of the HPLC. The samples were injected into a Shimadzu LC-10A HPLC system according to the method described by Wright *et al.* (1991), although the linear gradient was modified slightly: 0 min: 100% A, 2 min: 100% B, 2.6 min: 90% B/10% C, 13.6 min: 65% B/35% C, 20 min: 31% B/69% C, 28 min: 100% B, 31 min: 100% A. The HPLC system was calibrated with pigment standards from DHI (Water and Environment, Denmark). Peak identities were routinely confirmed by diode array. We are not aware of algorithms and pigment ratios developed for freshwater periphyton (*e.g.*, Mackey *et al.* 1996). Hence, the group composition of periphyton was estimated from the concentration of diagnostic pigments. Presence and relative concentration of diatoms were detected by fucoxanthin, diadinoxanthin, and diatoxanthin; chlorophytes were detected by chlorophyll *b*, lutein, violaxanthin, and neoxanthin; and cyanobacteria were detected by zeaxanthin.

Results

Comparative Toxicity of Herbicides on Photosynthesis

Isoproturon, metribuzin, and hexazinone had distinct effects on photosynthetic activity, which was reduced at increasing concentrations, whereas pendimethalin did not show any effects (Figure 1). Duration of exposure affected the algae community differently: Exposure to isoproturon for 1 h had no effect at the lowest concentration, but after 24 h of exposure photosynthesis was reduced even at the lowest concentration used, *i.e.*, 0.4 $\mu g\ L^{-1}$. The NEC value was determined at 1.00 $\mu g\ L^{-1}$ for the periphyton exposure to isoproturon for 1 h, while the NEC was 0.019 and $<0.01\ \mu g\ L^{-1}$ in the tests with 24-h exposure with isoproturon (Table 1). Correspondingly, the calculated effect concentration (EC_{50}) of isoproturon was higher for 1-h exposure (11.3 $\mu g\ L^{-1}$) than after 24-h exposure (1.74 and 0.53 $\mu g\ L^{-1}$, respectively) (Table 1). The response to hexazinone during 1-h exposure stimulated photosynthesis at the three lowest concentrations (0.4, 2, and 10 $\mu g\ L^{-1}$, Figure 1), and NEC and EC_{50} could not be determined. The stimulating effect was not found after 24 h exposure (Figure 1), when NEC of hexazinone was 2.29 $\mu g\ L^{-1}$, and EC_{50} was relatively high, *i.e.*, almost 33 $\mu g\ L^{-1}$ (Table 1). Metribuzin was highly toxic to the periphyton at low concentrations; NEC was 0.11 $\mu g\ L^{-1}$ and EC_{50} was 5.57 $\mu g\ L^{-1}$ after 24 h exposure (Table 1, Figure 1). Unfortunately, effect concentration for periphyton exposure 1 h for metribuzin was missing in this experiment, and effects of exposure duration of was investigated in a new experiment in May 2000.

Effect of Exposure Time Within Metribuzin

The periphyton community sampled in May 2000 was less sensitive to metribuzin in their photosynthetic response than the community sampled in September 1999. NEC increased from 0.11 $\mu g\ L^{-1}$ in September to 2.35 $\mu g\ L^{-1}$ in May, and EC_{50} was 5.57 $\mu g\ L^{-1}$ in September and 15.23 $\mu g\ L^{-1}$ in May after 24 and 23 h exposure, respectively (Tables 1 and 2). The metribuzin treatment had even (in some instances) a stimulating effect on photosynthesis in May at the lowest concentrations (Table 1 and Figure 2). EC_{50} varied during the exposure period between 9.6 $\mu g\ L^{-1}$ and 34 $\mu g\ L^{-1}$ (Table 2) and was on average 24 ± 10 (SD), and there was no trend of increasing or decreasing toxicity as a function of the duration of exposure in this experiment (Figure 2, Table 2).

The photosynthetic activity of the periphyton recovered almost completely 48 h after transferring to herbicide-free water. The effects of metribuzin on the photosynthetic activity were only short-term and apparently reversible (Figure 3).

Effect of Metribuzin on Pigment and Algal Group Composition of Periphyton Communities

The pigments detected at start indicated that especially diatoms (*i.e.*, fucoxanthin, diadinoxanthin, and diatoxanthin) and chlorophytes (*i.e.*, chlorophyll *b*, lutein, violaxanthin, neoxanthin) were abundant on the glass discs (Figure 4). Also cyanobacteria (*i.e.*, zeaxanthin) were present, although in lower abundances when comparing the concentrations of the algal pigments on the discs.

Metribuzin distinctly affected the pigment concentrations of the periphyton communities (Figures 5 and 6). Metribuzin reduced chlorophyll *a* (Chl *a*) concentration at all tested con-

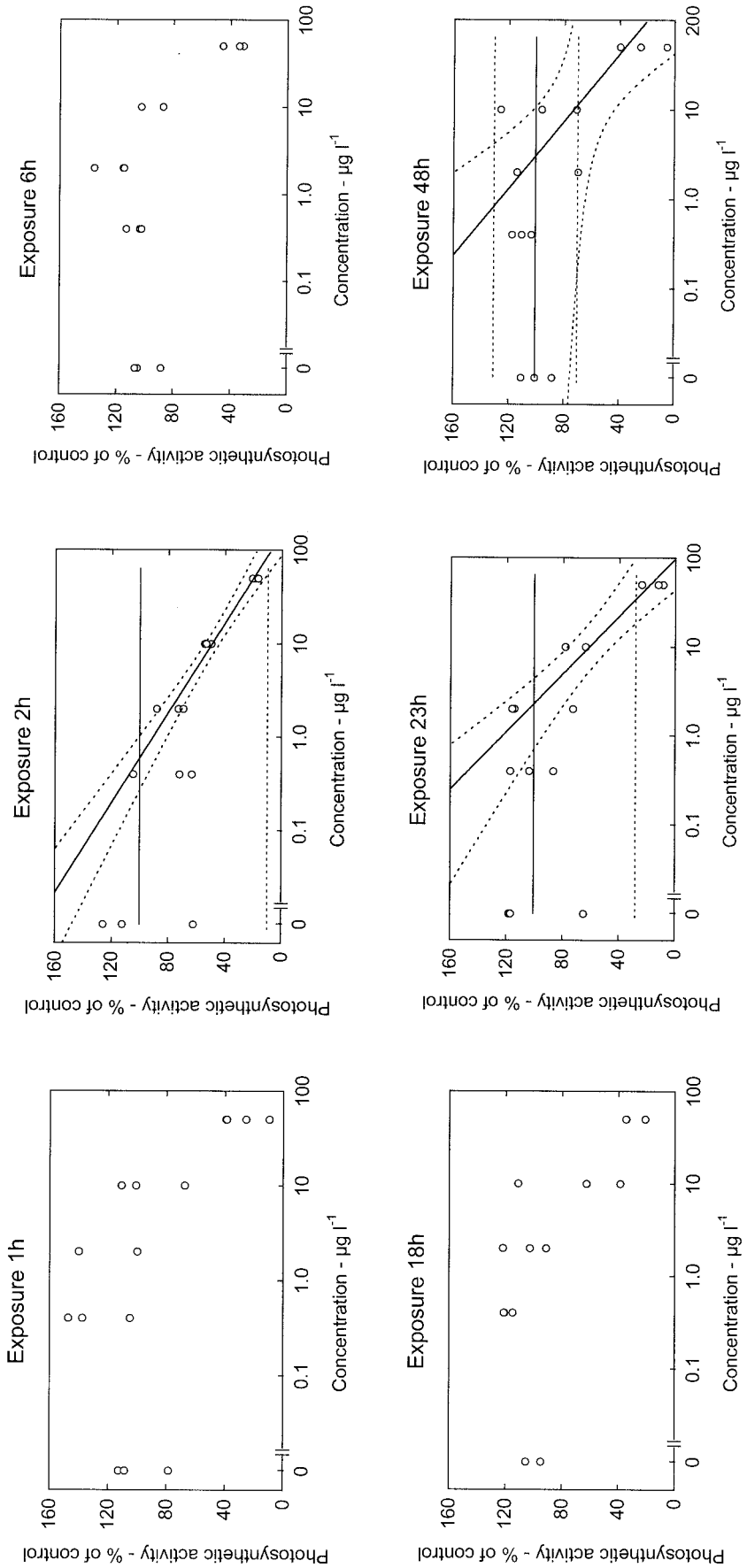

Fig. 2. Effect of metribuzin on the photosynthetic activity of periphyton communities. Effect was investigated after 1, 2, 6, 18, 23, and 48 h exposure for metribuzin. Dotted lines indicate 95% confidence limits of the controls and the linear regression

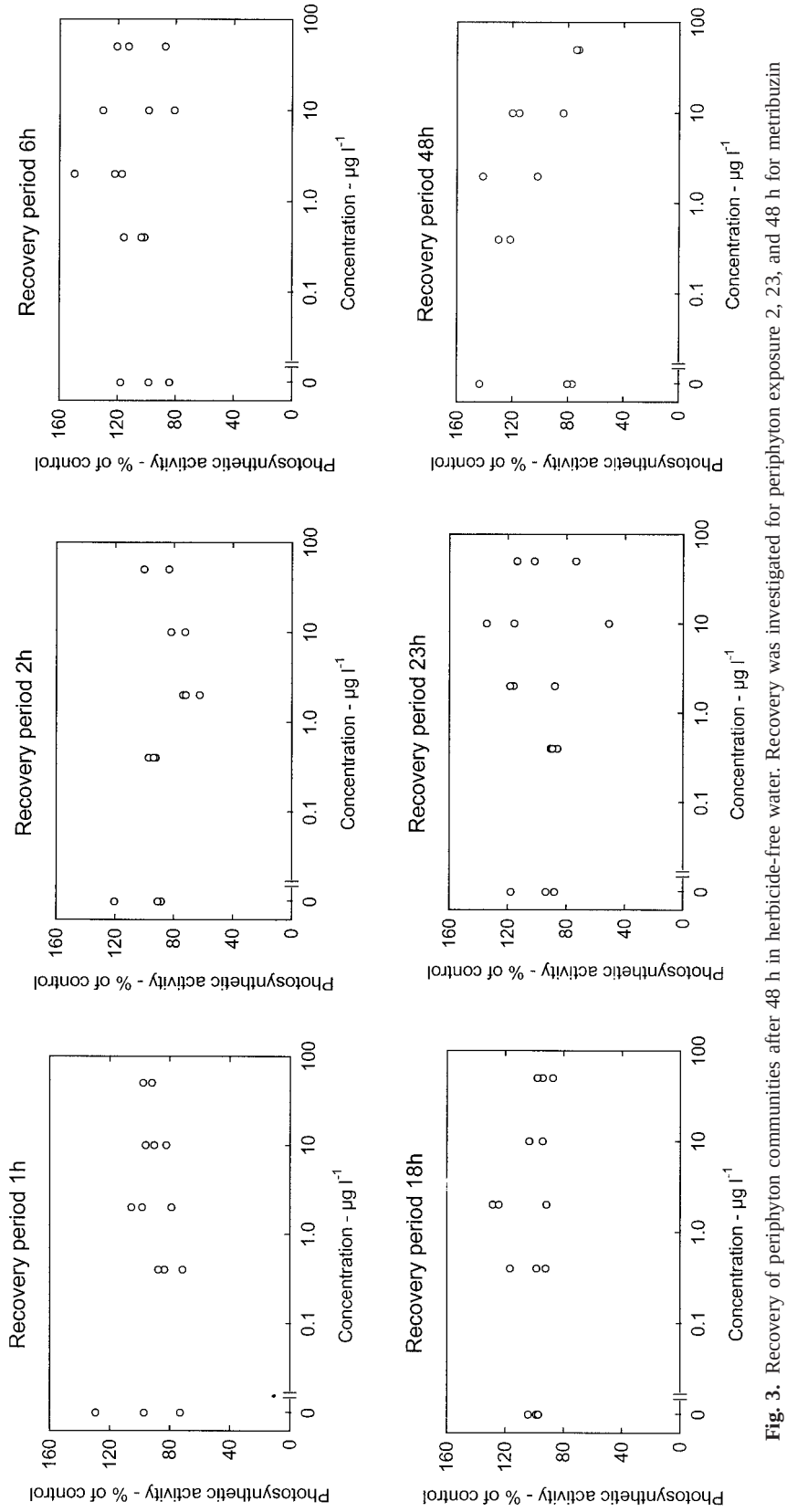

Fig. 3. Recovery of periphyton communities after 48 h in herbicide-free water. Recovery was investigated for periphyton exposure 2, 23, and 48 h for metribuzin

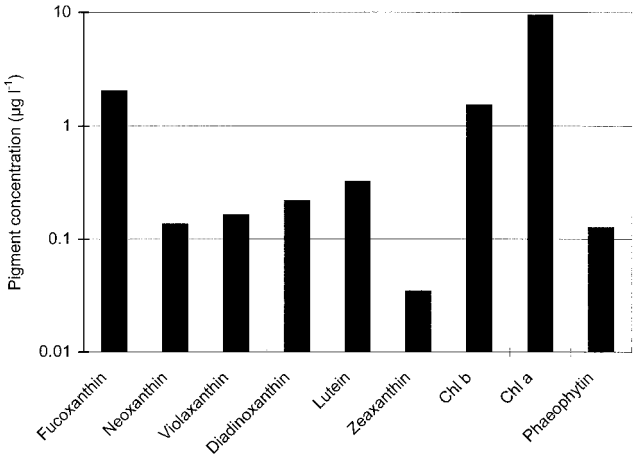

Fig. 4. Pigment composition of periphyton communities (start value) used in investigation for effect of exposure duration of metribuzin exposure and recovery

centration in a consistent pattern. At the highest concentration (50 μg L^{-1}) Chl *a* concentration was reduced about 30% after 23 h and 70% after 48 h. Besides, metribuzin affected the concentration of diagnostic pigments and the associated periphyton groups differently. The concentration of fucoxanthin and zeaxanthin increased slightly relative to controls after 23 h at 0.4, 2, and 10 μg metribuzin L^{-1}. After 48 h exposure, higher concentration of fucoxanthin, neoxanthin, violazanthin, lutein, zeaxanthin, and Chl *b* relative to controls was found at low and intermediate concentration of metribuzin. Changes in the diagnostic pigments fucoxanthin, zeaxanthin, and Chl *b* indicate group displacement in periphyton. Chlorophytes (detected by Chl *b*, lutein, violaxanthin, and neoxanthin) in particular were negatively influenced by exposure to metribuzin, whereas cyanobacteria (detected by zeaxanthin) and diatoms (detected by fucoxanthin and diadinoxanthin) in particular apparently were the groups responsible for the general increase in Chl *a* at lower metribuzin concentrations because they increased at all concentrations except at the highest concentration, 50 μg L^{-1} (Figure 7).

During the recovery period the Chl *a* concentration increased in all experiments, and although Chl *a* was affected by metribuzin during the exposure period, the periphyton algae resumed biomass at all concentrations after transfer to a herbicide-free medium (Figure 7). Only at the highest metribuzin concentrations after 48 h was the Chl *a* concentration still reduced in the recovery experiments compared to the lower concentrations, but had increased from 6 μg Chl *a* L^{-1} to 15 μg Chl *a* L^{-1} during the 48 h of recovery.

After transfer to clean water, both diatoms and especially cyanobacteria recovered well, *i.e.*, there was no difference in the concentration in the controls compared to the metribuzin treatments, and only diatoms were affected at 50 μg L^{-1} after 24 and 48 h exposure. The chlorophytes, however, were affected at all metribuzin concentrations, even after 48 h in clean water, also after only short-term exposure to metribuzin (Figure 7).

Discussion

Periphyton organisms are highly relevant as test organisms in toxicity tests because they constitute the most important food item for benthic fauna in streams, and effects on the productivity and biomass of periphyton caused by toxic substances may have an impact on the entire food web. Bonilla *et al.* (1998) found in experiments with episammon (microalgae on sand grains), periphyton, and phytoplankton that the different algal communities showed similar sensitivity to the herbicide simazine but different sensitivity to the herbicide paraquat, where periphyton was the most sensitive.

Generally, little data for effects of the selected herbicides on periphyton communities have been published. In the present study isoproturon affected photosynthesis activity severely at quite low concentrations. NEC in the 24-h test was below 0.02 μg L^{-1}, the LOEC was 0.4 μg L^{-1} (Figure 1), and EC_{50} was the lowest detected in these experiments, *i.e.*, 0.53–1.74 μg L^{-1} (Figure 1, Table 1). In a mesocoms experiment, diatom density and diversity in periphyton was reduced at the lowest concentration tested, 5 μg L^{-1} (Pérès *et al.* 1996). All values are distinctly lower than EC_{50} values for isoproturon published for standard single-species growth tests over 72 or 96 h: EC_{50} between 12–40 μg L^{-1} has been published for the planktonic micro algae *Chlorella pyrenoidosa*, *Scenedesmus subspicatus*, and *Chlamydomonas reinhardtii* (Traunspurger *et al.* 1996; Anton *et al.* 1993). These results illustrate the difficulty to predict what the effects under natural conditions will be from the standard single-species toxicological tests, because these tests do not include influences that occur on the community level, for instance, displacements in species composition, competition between species, and so on.

Compared to the frequent occurrence and concentration of isoproturon detected in Danish and Swedish streams, the effect concentration for periphyton is low. In Danish streams (Lillebæk and Odder Bæk) isoproturon is found in about 25% of the water samples and in concentrations between 0.011–0.068 μg L^{-1} (NERI 2002). In Swedish streams isoproturon has been detected in concentrations up to 10 μg L^{-1} (Kreuger 1998).

Metribuzin was also highly toxic to the periphyton community: In the experiment in September 1999, NEC was 0.11 μg L^{-1} and EC_{50} was 5.57 μg L^{-1} in 24-h t test (Table 1, Figure 1). All values are distinctly lower than EC_{50} values for metribuzin published for standard single-species growth tests over 96 h: EC_{50} for the planktonic micro algae *Chlorella* 31 μg L^{-1}, *Scenedesmus* 152 μg L^{-1}, *Selenastrum* 43 μg L^{-1}, *Chlamydomonas* 23 μg L^{-1}, *Microcystis* 100 μg L^{-1}, and *Anabaena* >3,000 μg L^{-1} (Fairchild *et al.* 1998). This value is distinctly higher than the effect concentration found in the present study in September 1999 for periphyton communities.

The effect concentration (EC_{50}) of isoproturon and metribuzin decreased by one to two orders of magnitude when the exposure time increased from 1–2 h to 24 h (Tables 1 and 2). These results imply that the effects of pesticides on periphyton communities largely depend on the duration of the exposure to the herbicides. This result is important to take into account in the risk evaluation of pulses of pesticides. This was also the case for hexazinone, although the effect concentration could not be estimated for the short-term exposure experiment because of inconsistency in the dose-response curves and lack of

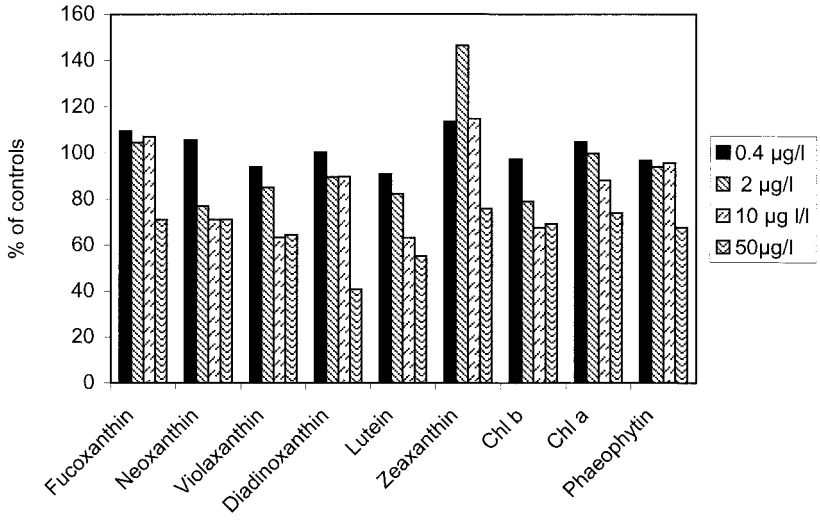

Fig. 5. Pigment composition of periphyton communities after 24 h exposure for metribuzin 0.4, 2, 10, and 50 µg L^{-1}. All values are in percent of controls

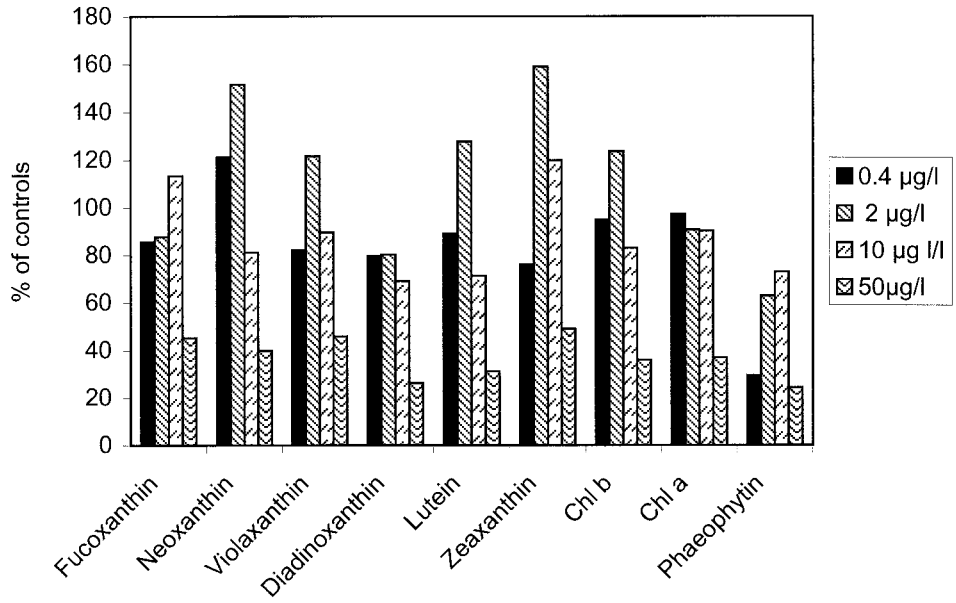

Fig. 6. Pigment composition of periphyton communities after 48 h exposure for metribuzin 0.4, 2, 10, and 50 µg L^{-1}. All values are in percent of controls

points in the calculation of NEC and EC_{50}. The inconsistency was caused by stimulation at the three lowest concentrations (0.4, 2, and 10 µg L^{-1}, Figure 1) of hexazinone compared to controls and inhibition at the highest concentration. After 24 h of exposure to hexazinone the stimulation of the photosynthesis disappeared (Figure 1), the dose-response curve was consistent, and NEC and EC_{50} for hexazinone were 2.29 and 32.88 µg L^{-1} (Table 1). Such stimulation in a short-term experiment is commonly found as a short-term response to toxic stress. However, the stimulation is obviously a response to the toxicant, and it is difficult to evaluate its effect.

Pendimethalin did not show any effects on the natural community of periphyton at concentrations up to 50 µg L^{-1}. The solubility of pendimethalin in water is relative low, *i.e.,* about 275 µg L^{-1}, and pendimethalin has a relatively high affinity for particles, both properties that indicate that the bioavailability for algae may be low. Swedish investigations indicated that the transport of pendimethalin from agriculture to streams is probably small (Kreuger 1998). In contradiction to the Swedish results, pendimethalin has been found in Danish stream in concentrations of up to 0.077 µg L^{-1} (NERI 2002).

Metribuzin affected the periphyton sampled in September 1999 and in May 2000 differently. The community sampled in May was less sensitive to metribuzin than the community sampled in September, and photosynthesis in May was even stimulated at the lowest concentrations (Tables 1 and 2, Figures 1 and 2). These results confirm the hypothesis that natural communities are highly variable due to influence by physical, chemical, and biological parameters, and toxic substances have different impacts on and result in different effect concentrations of the algal communities depending on season, species composition, nutrient status, etc. (Guasch *et al.* 1997). Unfortunately, the group composition was not analyzed in September. Communities typically consist of many different species that differ largely in sensitivity to toxicants. The difference in sensitivity found may be explained by the dominance of me-

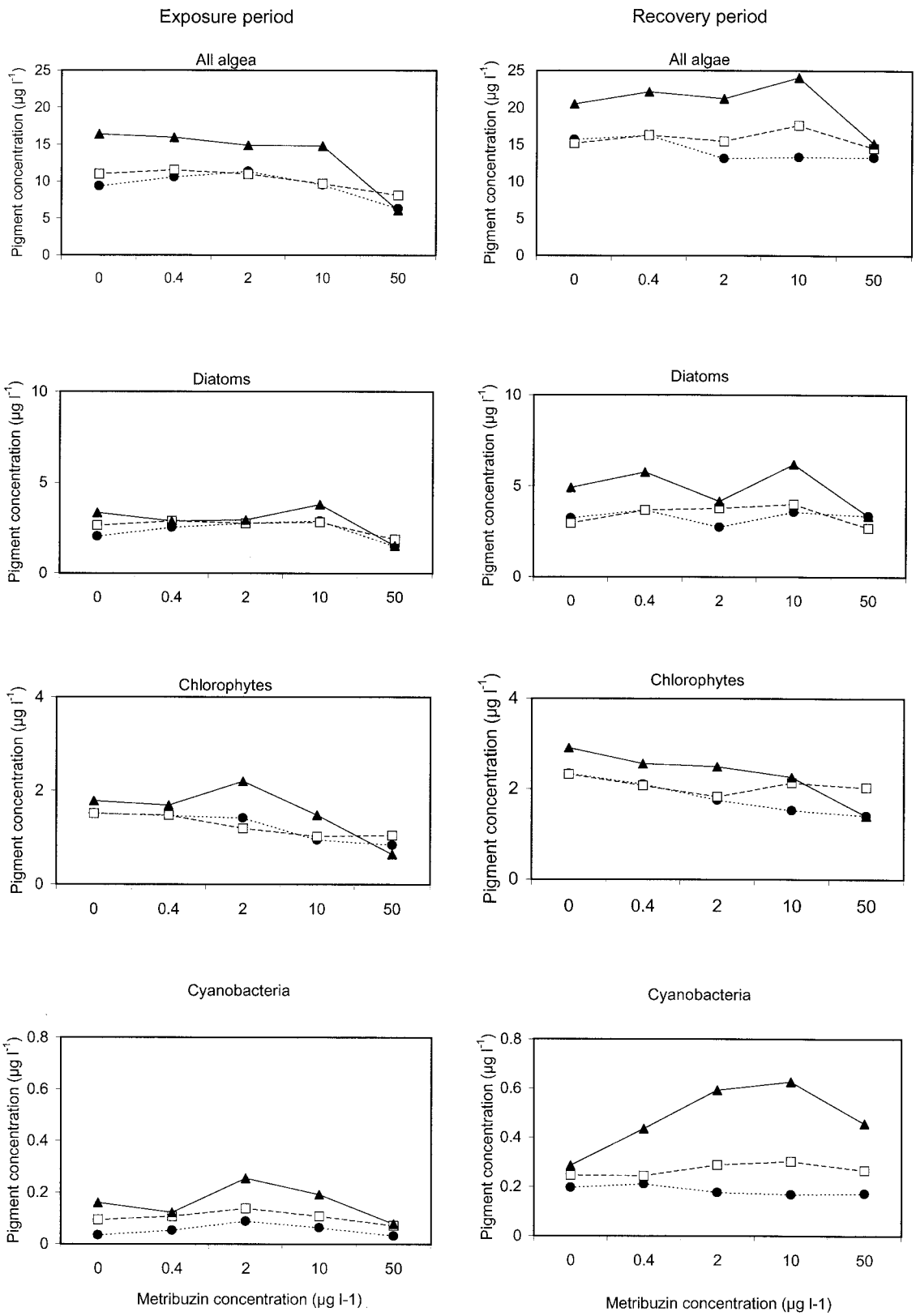

Fig. 7. Effect of metribuzin on the different periphyton groups were measured after 2, 23, or 48 h of exposure and following recovery after 48 h in herbicide-free water. The diagnostic pigments used were: all algae: chlorophyll *a;* diatoms: fucoxanthin; chlorophytes: chlorophyll *b;* and cyanobacteria: zeaxanthin. Group composition is investigated on community after, respectively 2 h (circles), 23 h of exposure (squares), and 48 h exposure for metribuzin (triangles).

tribuzin-tolerant/sensitive species in the communities sampled in May. In the case that tolerant species dominated the community the risk of the toxicant could be underestimated. Because of the variations in the sensitivity of phytoplankton communities at different locations and at different times of the year, it has been advocated that community tests with phytoplankton are unsuitable for hazard evaluations of toxicants (Kusk and Nyholm 1991). However, single-species tests are not a good alternative because variability in sensitivity for toxicants may differ by up to three orders of magnitude between the different species, and no general sensitive algae species have been identified (Blanck *et al.* 1984; Wängberg and Blanck 1988; Källqvist and Romstad 1994; Fairchild *et al.* 1997, 1998).

In the recovery experiment following metribuzin exposure, EC_{50} did not reveal any increasing or decreasing trend in toxicity as a function of the duration of the exposure but varied in a range between 9.57 and 34.00 $\mu g\ L^{-1}$. NEC and LOEC did not differ much throughout the 48-h exposure experiment (Figure 3, Table 2). The inhibition of the photosynthesis due to metribuzin exposure was therefore not dependent on the duration of the exposure to this toxicant. This was also found in the study by Bonilla *et al.* (1998) in experiments with natural communities of microalgae and the herbicide simazine, while the inhibition due to exposure to the herbicide paraquat was dependent on the exposure time, which increased from 18% to 76% between 30 min and 24 h of exposure.

The recovery of photosynthetic activity was almost complete after 48 h in herbicide-free water even at the highest concentrations, for which metribuzin had inhibited the photosynthetic activity by 80%. This result indicates that the effect of metribuzin on periphyton is reversible even at very high concentrations. However, this was not the case because the composition of the periphyton long-term was affected even by short-term exposure (2 h) at the lowest concentration tested (0.4 $\mu g\ L^{-1}$).

Metribuzin is a triazine herbicide that may inhibit transport of electrons in photosystem II and the chloroplast (Hassall 1990). Triazine is known to induce the so-called greening response, similar to shade adaptation where chlorophyll content increased (*e.g.,* Koenig 1990). However in our investigation metribuzin reduced Chl *a* concentrations at all tested concentrations, and our result did not indicate any greening effects in either the exposure or recovery period. On this background we conclude that the overall changes in concentration and composition of pigment in the periphyton communities is related to changes in biomass and composition of different algal group (Figures 5 and 6).

Because the concentration of accessory pigments is related to the Chl *a* concentration (Schlüter *et al.* 2000), the change in the concentrations of the detected pigments can be used for assessing the development of the biomass of the respective groups as effects of the treatment with the herbicide metribuzin. For chlorophytes and diatoms, several specific pigments were detected (neoxanthin, violaxanthin, lutein [chlorophytes], and diadinoxanthin and diatoxanthin [diatoms], data not shown), and the development of these pigments closely followed the diagnostic pigments, Chl *b* and fucoxanthin, respectively, indicating that this method is very robust. The chlorophytes were the group most affected by exposure to metribuzin, and they were almost always reduced due to the metribuzin treatment, while especially cyanobacteria but also diatoms at the lowest concentrations, *i.e.,* 0.4, 2, and 0 $\mu g\ L^{-1}$ (Figure 7), were stimulated due to the metribuzin exposure. This result is in agreement with single-species data that indicate that cyanobacteria compared to chlorophytes may be very tolerant for metribuzin (Fairchild *et al.* 1998). This gives evidence of a different response to metribuzin within the periphyton community, where the negative impact on chlorophytes and positive impact on diatoms and cyanobacteria causes displacements in the composition of the algae community within 48 h. Displacements in the biomass of such functionally different algae groups result in changed food availability and quality for the grazers, which ultimately will have impact on the entire food web. At the highest applied metribuzin concentration, 50 $\mu g\ L^{-1}$, the biomass of all periphyton groups was reduced compared to the control. Consequently, potential indirect impact on higher trophic levels of metribuzin and other pesticides, which may affect the composition and biomass of periphyton, warrants further investigation.

The induced changes in composition of the communities and the fast recovery of the photosyntethic activity are in good agreement to the responses found in other studies including the effects of toxicants on algae communities, *e.g.,* tributyltin (Blanck and Dahl 1996; Petersen and Gustavson 1998), atrazine (Gustavson and Wängberg 1995), and arsenate (Blanck and Wängberg 1988). The most likely direct effects of herbicides on periphyton communities in streams may be the exclusion and inhibition of sensitive species.

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