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Environmental Toxicology and Chemistry, Vol. 22, No. 6, pp. 1262–1268, 2003
Printed in the USA
0730-7268/03 $12.00 + .00

Environmental Toxicology

METHYL PARATHION TOXICITY IN VEGETATED AND NONVEGETATED WETLAND MESOCOSMS

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(*Received* 18 *June* 2002; *Accepted* 1 *September* 2002)

Abstract—Methyl parathion (MeP) was introduced into constructed wetlands for the purpose of assessing the influence of emergent vegetation on transport and toxicity of the pesticide. Two vegetated (90% cover, mainly *Juncus effusus*) and two nonvegetated wetland cells (each with a water body of 50 × 5.5 × 0.2 m) were each dosed with 6.5 m^3 of water containing active ingredient of MeP at 6.6 mg/L associated with suspended soil at 400 mg/L to simulate a storm runoff event. Acute toxicity was assessed by sampling benthic macroinvertebrates at 5, 10, 20, and 40 m from the inlet before and 96 h after contamination and by in situ exposure of *Chironomus tentans* (Diptera) up to 24 h after contamination. Methyl parathion was detected throughout the nonvegetated wetland cells (70 μg/L at 20 m, 8 μg/L at 40 m), whereas the pesticide was not transported through the vegetated wetland cells (20 μg/L at 20 m, <0.1 μg/L at 40 m). A three-way analysis of variance using contamination (repeated measure variable), location, and vegetation indicated significant negative effects of contamination on various insect taxa, such as mayfly nymphs and caddisfly larvae. Seven out of the total of 15 species revealed a significant contamination × vegetation effect, with individuals in the vegetated wetlands being less affected. Four species showed a significant contamination × location effect, confirming a higher toxicity in the inlet area of the wetlands. A significant three-way interaction of contamination × vegetation × location was detected in *Chironomus* sp., which was most strongly affected at the inlet area of the nonvegetated wetland cells. The in situ bioassay employing *C. tentans* confirmed the positive effect of wetland vegetation on MeP toxicity. These results demonstrate the importance of vegetation for pesticide mitigation in constructed wetlands.

Keywords—Insecticides Risk mitigation Non–point-source pollution Vegetation Wetland communities

INTRODUCTION

Constructed wetlands recently have been shown to have the ability to retain non–point-source insecticide pollution and preventing it from entering receiving aquatic habitats [1–3]. The implementation of retention ponds in agricultural watersheds was mentioned by Scott et al. [4] as one strategy to reduce the amount and toxicity of runoff-related insecticide pollution discharging into estuaries. The usefulness of aquatic plants for removal of insecticides from water has been shown [5] and the effects of the organophosphate phorate have been assessed by using littoral mesocosms in South Dakota (USA) wetlands [6]. However, few other studies in the open literature deal with the fate or effects of agricultural insecticide input in constructed wetlands.

Processes important for removal of non–point-source pesticide runoff in wetlands may include adsorption, decomposition, and microbial metabolism [7]. The macrophytes present in the wetland may play an important role in providing an increased surface area for sorption as well as for microbial activity [8,9]. Furthermore, they may contribute directly to chemical metabolism [10]. Emergent vegetation was demonstrated to reduce the resuspension of sediments in wetlands [11].

Spray drift and runoff are important routes for non–point-source pesticide pollution of aquatic habitats, and runoff has been shown to possibly contribute to greater concentrations and loads of insecticides than spray drift [12]. Runoff is the major source of aquatic insecticide input in the intensively cultivated Mississippi River (USA) delta region [13]. Constructed wetlands could serve as a suitable risk mitigation strategy for agricultural runoff, given that enough information on their effectiveness with specific reference to the importance of the wetland vegetation is available.

Biological effects of pesticides in wetlands have been studied under experimental conditions with mesocosms [14], in littoral enclosures [6], or in the field by employing organisms in situ [1,2,4]. The need now exists to link wetland characteristics such as the presence of emergent macrophyte vegetation with the transport of non–point-source pesticide contamination and the resulting biological effects. The following study was undertaken for this purpose. Methyl parathion, an organophosphate insecticide primarily applied to cotton, was chosen as the test substance for a simulated runoff event. The use of MeP in the lower Mississippi River delta averages approximately 400,000 kg of active ingredient per year [15] and MeP has been detected at high levels in agricultural runoff [16]. Methyl parathion has an organic carbon partition coefficient (K_{OC}) of 5,100 and a water solubility of 55 mg/L.

MATERIALS AND METHODS

Description of the wetland mesocosms

Constructed wetlands (water body: 50 × 11 × 0.2 m) at the University of Mississippi Field Station (Abbeville, MS,

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USA) were specifically designed to evaluate the fate of pesticides in wetlands [3]. Four of these constructed wetlands, orientated in parallel and independent from each other in terms of water supply, were used for this research. Two wetlands differing in vegetation coverage were chosen as experimental cells. The vegetated wetland had a macrophyte coverage of more than 90% (*Juncus effusus*: 171 ramets/m^2 and *Leersia* sp.: 12 ramets/m^2) and the nonvegetated wetland had a macrophyte coverage of less than 5%. Both wetlands were divided longitudinally 5 d before contamination, so that each comprised two wetland cells (water body: 50 × 5.5 × 0.2 m); the divider consisted of metal flashing (50 m × 0.5 m × 1 mm) that was pressed 10 cm into the wetland sediment. The two remaining wetlands were used as water sources for the simulated storm event. Above-surface platforms at 5, 10, 20, and 40 m from the inlet were used in each wetland cell to ensure that sampling could be done without the necessity to walk into the wetlands, which may destroy the macrophytes, sediments, or both. The total number of sampling sites was 16 (two nonvegetated and two vegetated wetland cells with four stations each).

Experimental procedure and pesticide analysis

Each of the four wetland cells was treated at the inlet with MeP in a soil–water mixture to simulate agricultural runoff on August 11, 2000. The amount of MeP applied as simulated runoff was based on assumptions of an immediate (postapplication) 6.35-mm rainfall on 50-ha agricultural fields to which commercial grade MeP (Clean Crop®, United Agri Products, Greely, CO, USA) at a rate of 8.6 kg active ingredient per 20 ha had been applied. Based on the assumption of 1% pesticide runoff [16], a total of 43 g active ingredient of MeP in a volume of 6,500 L of water was added to each of the four wetland cells. Additional inclusion of 2.5 kg sandy loam (84% sand; 16% silt) per wetland cell was designed to simulate the typical suspended solid load (400 mg/L) in the Mississippi Delta Ecoregion. An amount of 100 L of water per wetland was mixed with soil and pesticide in a mixing chamber and was introduced into runoff water during the 30-min contamination period. The soil and pesticide mixture was homogenized for a 24-h period before the experiment. The surface velocity through the wetlands during runoff simulation was below 0.05 m/s.

Water samples for pesticide analysis were taken at 3 h, 6 h, 24 h, 96 h, and 10 d after application at the 16 sampling stations. Solvent-washed 1,000-ml amber glass bottles were used to collect aqueous samples. After collection, samples were placed on ice (< 2 h) until transported to a walk-in cooler (4°C) pending analysis. Sample extraction and analysis were as outlined in Moore et al. [17]. Sediment and subsurface plant samples were taken at 24 h after application and analyzed as documented in Bennett et al. [18]. All samples were analyzed via gas chromatography–microelectron capture detection with a Hewlett-Packard 6890 gas chromatograph equipped with a DB5 MS column (Hewlett-Packard, Avondale, PA, USA). The limits of detection for MeP in water, sediments, and plants were 0.001 μg/L, 0.02 μg/kg dry weight, and 0.02 μg/kg dry weight, respectively. Based on fortified samples, mean extraction efficiencies were >90%.

The mean values (n = 3) for pH, dissolved oxygen, temperature, and conductivity in the vegetated and nonvegetated wetland were 6.7 ± 0.1 and 6.9 ± 0.2, 2.3 ± 1.0 mg/L and 6.6 ± 0.7 mg/L, 25.2 ± 1.3°C and 27.8 ± 0.5°C, and 116 ± 6.5 μS/cm and 43 ± 3.4 μS/cm, respectively.

Macroinvertebrate sampling and in situ exposure bioassays

Sampling of macroinvertebrates was performed 2 d before contamination and 96 h after contamination. Four samples were taken at each of the 16 sites and each of the two dates by using an Ekman sampler (area = 15 × 15 cm). Samples were transported within 1 h to the laboratory, sorted out in white plastic tubs, preserved in 70% ethanol, and determined to species or genus level with dissecting microscopes.

Midges (*Chironomus tentans*) were used as a test organism. Animals were obtained from a culture at the Ecotoxicology Research Facility at Arkansas State University (State University, AR, USA). At each of the 16 sites, four replicate exposure beakers containing 10 fourth-stage larvae were installed 1 h before contamination. The number of surviving larvae was counted at 3 and 24 h after exposure. The in situ exposure methodology is outlined in detail in Schulz et al. [19].

Data analysis

Linear regression analyses were used to fit curves to base 10 log-transformed MeP water concentrations (y) versus distance downstream of the pesticide inlet (x). Only the maximum concentrations (of the means from n = 2 samples) observed at each sampling distance (regardless of time) were used in the analysis. Biological data obtained for the two nonvegetated and vegetated wetland cells correlated well (r^2 = 0.94; p < 0.0001; df = 255) and thus were combined. Unfortunately, pseudoreplication is unavoidable in studies of the type undertaken here; hence, it is difficult to assess or exclude the effect of unmeasured or unknown covariables [20]. However, with regard to the size of the wetland mesocosms, the invertebrate samples taken at one site are regarded as sufficiently independent to justify an analysis of variance. Effects of vegetation (vegetated vs nonvegetated, factorial), location (5, 10, 20, and 40 m from the inlet, factorial), and contamination (before vs 96 h after MeP introduction, repeated measure variable) on the abundances of macroinvertebrates were analyzed by using a three-way analysis of variance (ANOVA). Similarly, the survival of in situ–exposed *C. tentans* was analyzed by using time (3 and 24 h after MeP introduction) as a repeated measure variable instead of contamination as with the community data. Abundance and survival data were transformed by using $\ln(x + 1)$ to satisfy the assumptions of ANOVA. We applied a Bonferroni correction to control for type I statistical errors and assessed statistical significance with α = 0.012.

RESULTS AND DISCUSSION

Methyl parathion concentrations

Maximum observed MeP concentrations in water were inversely proportional to the distance from the inlet (Fig. 1). However, the transport of MeP through the wetlands differed greatly depending on the vegetation coverage. Peak levels in the nonvegetated wetland were as high as 70 μg/L at 20 m and 8 μg/L at 40 m from the inlet, whereas the respective values were 20 μg/L and <0.1 μg/L in the vegetated wetland (Table 1). At 5 and 10 m from the inlet, the contamination was generally higher, but did not differ greatly between the nonvegetated (550 and 120 μg/L, respectively) and the vegetated wetland (420 and 180 μg/L, respectively). Apart from the 20- and 40-m station in the nonvegetated wetlands, all MeP levels were at least five times higher between 3 h and

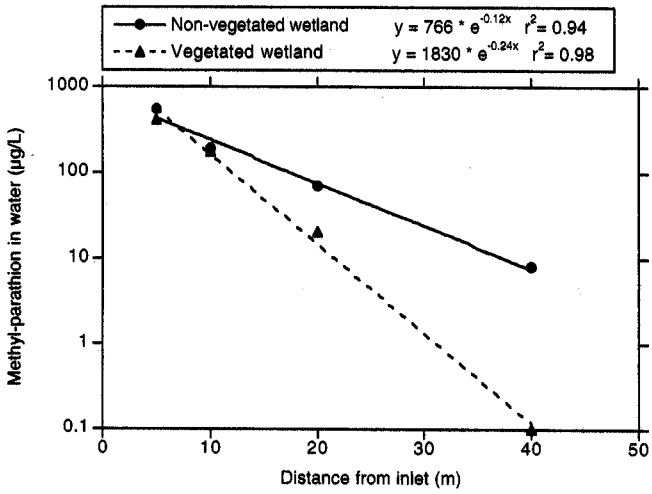

Fig. 1. Linear regression relationship between log-transformed maximum observed methyl parathion concentrations (detection limit: 0.1 µg/L) and distance downstream from the pesticide inlet.

24 h after application than at 96 h and at 10 d. On the basis of the pesticide added and the water volume of the wetland, a theoretical target concentration of 700 µg/L would result, assuming all the MeP to be in the water, immediate and equal mixing, and no degradation. As expected, the measured concentrations were generally lower than this target value. Sediment and plant samples taken at 24 h after application contained MeP concentrations up to 2,000 µg/kg dry weight and 10,000 µg/kg dry weight, respectively.

The pesticide analysis clearly revealed differences in the transport of MeP depending on the vegetation coverage of the wetland. The dense vegetation coverage in the vegetated wetland, with more than 180 ramets/m^2, likely leads to a reduced flow rate of the water through the wetland and the plant rhizomes may very well reduce the seepage velocity through the sediments [21] and thus reduce the pesticide transport. Similar processes are well known for nutrients [22] and suspended particles [23] but have been rarely demonstrated for insecticides. However, a role for aquatic plants in facilitating the removal of pesticides from the water via adsorption has been implicated by a number of workers [8,9]. The importance of sorption and partitioning of MeP in river biofilms has been demonstrated under experimental conditions by Headley et al. [24]. Our results concerning high MeP contents in the plants and sediments 24 h after application confirm the importance of sorption in reducing pesticide levels in the water.

Table 1. Methyl parathion concentrations in water samples (µg/L) taken at different distances from the inlet between 3 h and 10 d after application. Each value represents the mean of analysis of two separate samples taken from each wetland cell replicate (ND = not detectable, i.e., 0.1 µg/L)

	Distance from inlet (m)	Time after application				
		3 h	6 h	24 h	96 h	10 d
Nonvegetated	5	550	350	180	40	3
	10	120	190	160	30	4
	20	40	70	40	20	0.6
	40	0.3	1	6	8	0.6
Vegetated	5	420	300	190	20	4
	10	180	120	90	10	1
	20	10	20	10	1	ND
	40	ND	ND	ND	ND	ND

A half-life time of 12 h has been reported for MeP in water at initial levels of 200 µg/L when using 20-L glass aquaria in the laboratory [25]. Based on this half-life time, an initial concentration of 550 µg/L at the 5-m station in the nonvegetated wetland would be reduced to values below the detection limit (<0.1 µg/L) after about 7 d. The fact that levels between 0.6 and 4 µg/L were measured even after 10 d indicates that the degradation of MeP under high-temperature and low-oxygen conditions in the wetlands was much slower than would have been expected from the reported laboratory half-life times.

Macroinvertebrate community responses

A total of 15 species were found in the wetlands (Table 2). *Caenis latipennis* (Ephemeroptera) and *Chironomus* sp. (Diptera) were the most dominant species, forming more than 50% of the individuals before contamination. Six out of the 15 species were odonate species and 13 species belonged to the insect group. The macroinvertebrate composition is typical for static mud bottom ponds with low oxygen concentrations [26].

The abundances before and 96 h after the contamination are compared for the nonvegetated and vegetated wetland in Figure 2. A significant negative acute effect of contamination on abundances was found in 8 of the 15 species (Fig. 2 and Table 2), resulting also in a significant negative effect of contamination on the total numbers of individuals (Fig. 2p and Table 2). The two mayfly species and the caddisfly *Oecetis cinerascens* were no longer found after contamination (Fig. 2b, c, and k). The strong negative effects of MeP on the abundance of macroinvertebrates are in accordance with the fact that the measured concentrations were well above levels that are reported to be acutely toxic to aquatic invertebrates. The 24-h median effective concentration (EC50) for the cladoceran *Ceriodaphnia dubia* is 5.5 µg/L [27] and the 96-h median lethal concentration (LC50) for the damselfly *Ischnura verticalis* is 33 µg/L [28]. A high susceptibility of mayfly and caddisfly species to insecticide components has been reported in other studies [29]. A significant decline of mayfly abundance also was reported for experimental outdoor ponds treated with MeP at 100 µg/L [30].

Seven of the eight species that were affected by the contamination showed a stronger negative response in the unvegetated than in the vegetated wetland (Fig. 2), as indicated by the significant contamination × vegetation interaction in the ANOVA (Table 2). This interaction also was significant for the total number of individuals (Table 2). Because four of these eight species were not present in either the vegetated or the nonvegetated wetland before contamination, a direct comparison of the effects of contamination in relation to vegetation coverage is not possible for these species. However, *Chironomus* sp. was found in both wetlands and was affected to a significantly higher extent by contamination in the nonvegetated wetland (Fig. 2e and Table 2). *Caenis latipennis*, *Callibaetis* sp., and *O. cinerascens* were 100% eliminated from both wetlands after treatment (Fig. 2b, c, and k); however, these three species occurred at higher densities in the nonvegetated wetland than in the vegetated wetland before contamination.

The reaction of macroinvertebrates clearly demonstrated that the impact of MeP in the vegetated wetland was considerably lower than in the nonvegetated wetland. This result is

Table 2. Summary of the statistical significance from three-way analysis of variance (ANOVA) of the effect of vegetation ([veg.] vegetated vs nonvegetated), location ([loc.] 5, 10, 20, and 40 m distance from the inlet), and contamination ([cont.] before vs 96 h after methyl parathion introduction, repeated measure variable) on the abundances ($\ln(x + 1)$ transformed) of various macroinvertebrate species[a]

Species	Group[b]	Veg.	Loc.	Veg. × loc.	Cont.	Cont. × veg.	Cont. × loc.	Cont. × veg. × loc.
Branchiura sp.	C	NS	NS	NS	NS	NS	NS	NS
Caenis latipennis	E	***	NS	NS	***	***	NS	NS
Callibaetis sp.	E	*	NS	NS	***	*	NS	NS
Celina sp.	C	NS	NS	NS	NS	NS	NS	NS
Chironomus sp.	D	***	***	NS	***	***	***	*
Dromogomphus spinosus	O	*	***	NS	NS	NS	***	NS
Epitheca cynosura	O	NS	NS	NS	*	NS	**	NS
Ischnura verticalis	O	***	NS	NS	*	*	NS	NS
Libellula lydia	O	***	NS	NS	**	**	NS	NS
Lymnaea sp.	G	NS	NS	NS	NS	NS	NS	NS
Oecetis cinerascens	T	***	NS	NS	***	***	NS	NS
Pachydiplax longipennis	O	***	*	NS	NS	NS	NS	NS
Suphisellus sp.	C	**	NS	NS	NS	NS	NS	NS
Tabanus sp.	D	NS	NS	NS	NS	NS	NS	NS
Telebasis byersi	O	**	NS	NS	**	**	*	*
Total number of individuals		NS	***	NS	***	***	***	NS

[a] Significance levels: NS (not significant) $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.
[b] A = Annelida; C = Coleoptera; D = Diptera; E = Ephemeroptera; G = Gastropoda; O = Odonata; T = Trichoptera.

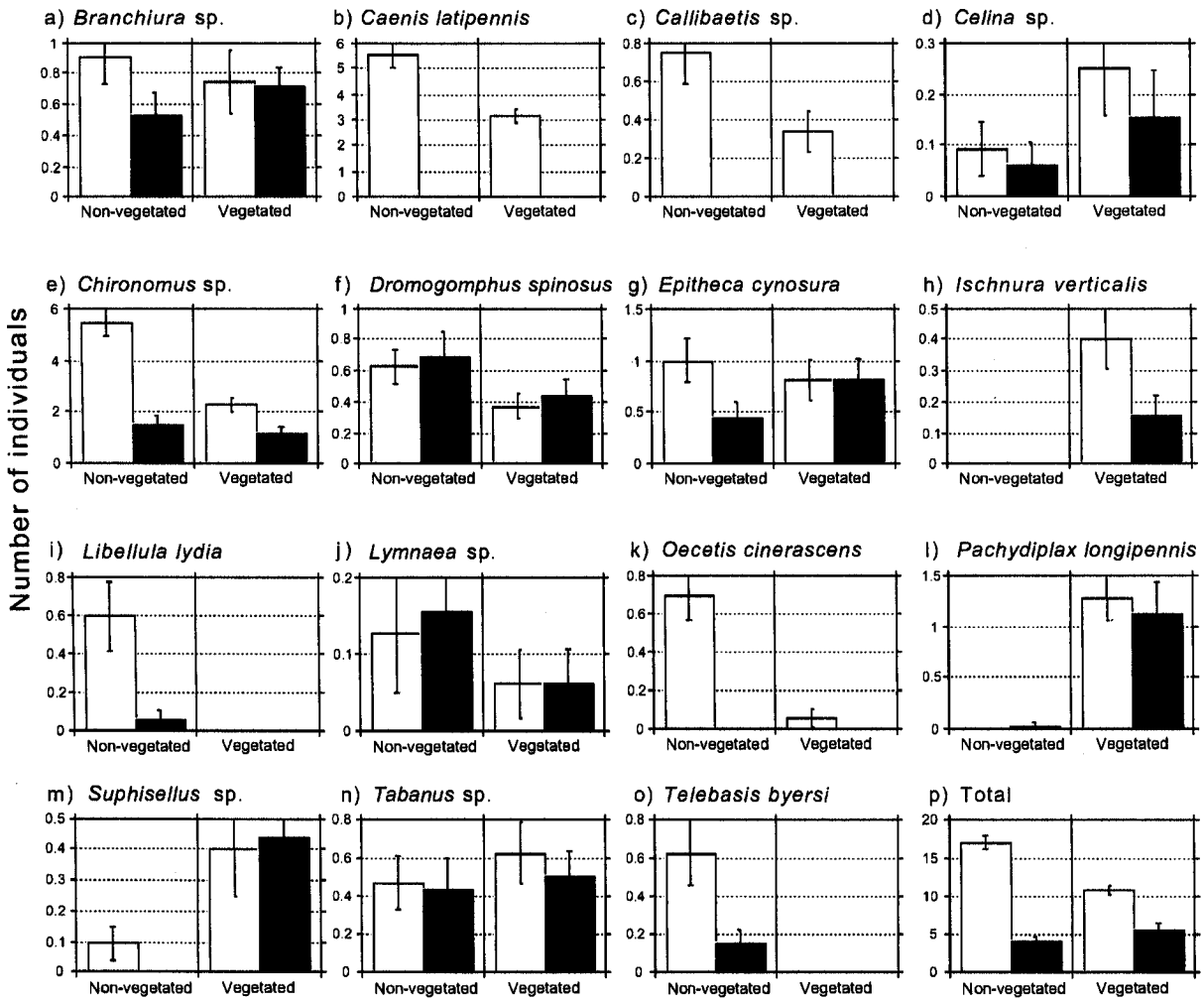

Fig. 2. Mean (± standard error, $n = 32$) abundance (individuals per sampler) of macroinvertebrate species in nonvegetated and vegetated wetland mesocosms before (white bars) and 96 h after (black bars) introduction of methyl parathion.

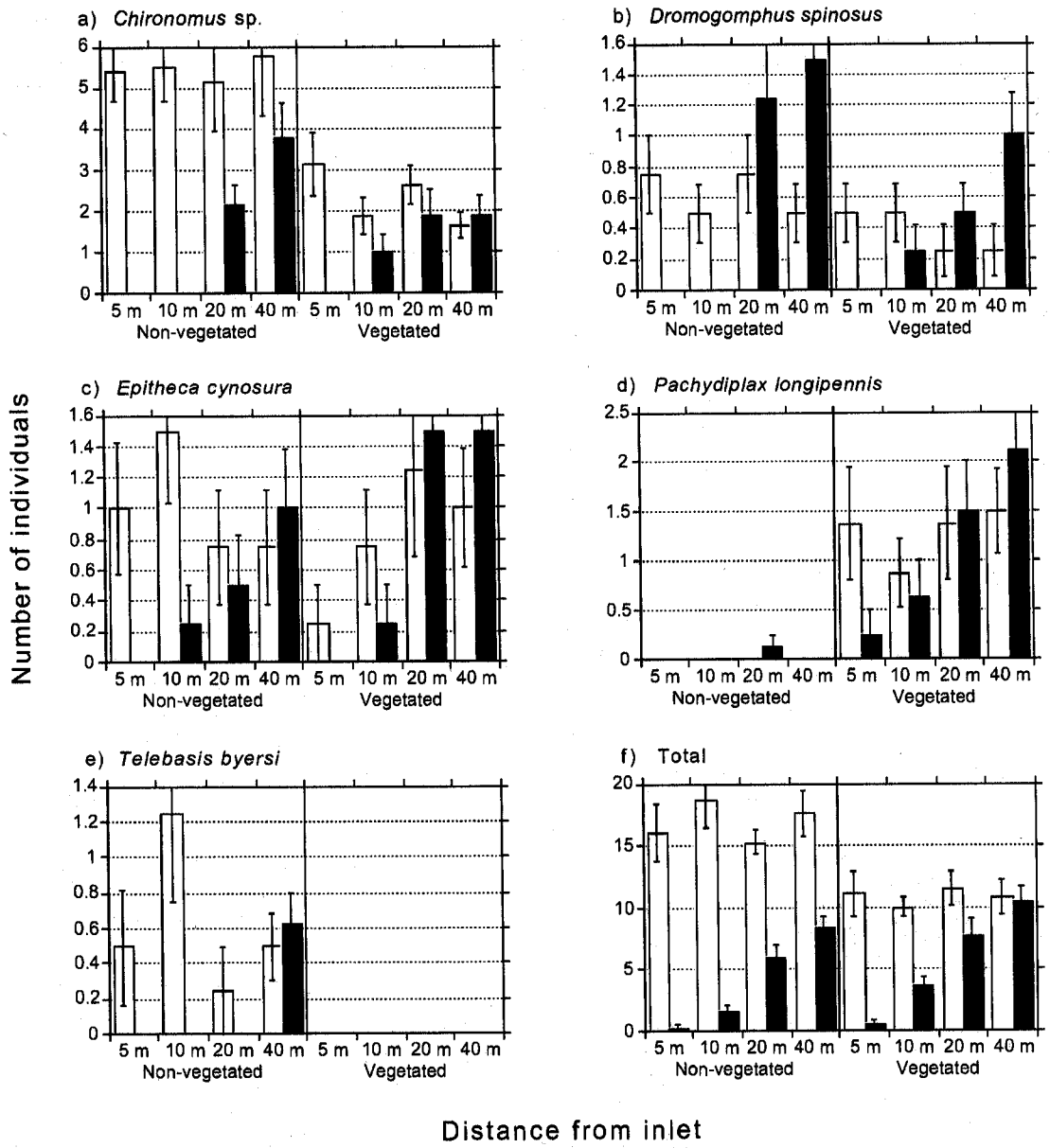

Fig. 3. Mean (± standard error, n = 8) abundance (individuals per sampler) of macroinvertebrate species in wetland mesocosms at different distances from the pesticide inlet station before (white bars) and 96 h after (black bars) introduction of methyl parathion.

in accordance with the observed differences in transport of MeP through the wetland and the resulting differences in exposure levels. Such a link of vegetation coverage in wetlands, pesticide transport, and toxicity to the inhabiting fauna has not been described yet in the open literature. However, input–output studies of constructed wetlands and retention ponds in agricultural watersheds recently demonstrated reductions of insecticide-associated toxicity [1,3,4]. The decreased toxicity in the vegetated wetland may result from a combination of reduced transport and increased sorption of the pesticide to the aquatic plants [8,9]. Differences in other water quality parameters would have been unlikely to contribute to the observed stronger effects in the nonvegetated wetland, because the oxygen concentrations were even lower, the temperature was only 2.6°C higher in the vegetated wetland, and the hardness was low in both wetlands.

Within both wetlands, a clear spatial gradient occurred in the abundances of various species. The abundances of 4 of the 15 species were reduced at a significantly higher rate near the inlet (5- and 10-m stations) than further away (20- and 40-m stations; Fig. 3a to c and e), which is indicated by the significant interaction for contamination × location from ANOVA (Table 2). The same contamination × location effect is present if the total number of individuals is taken into consideration (Fig. 3f and Table 2). The contamination × location effect is not significant for *Pachydiplax longipennis*, although this species showed a trend to be more affected near the inlet as well (Fig. 3d). The spatial differences in the biological reactions are again in accordance with the observed MeP distribution and thus further reinforce the inference that the pesticide is the cause of the changes in invertebrate abundances.

Four odonate species were present in even greater numbers at the 20- and 40-m stations after contamination (Fig. 3b to e), suggesting that they may have migrated within the wetland as a reaction to the MeP exposure. A spatial response of odonate larvae to environmental factors has been implicated by other workers [31,32]. *Chironomus* sp. showed a significant three-way interaction of contamination × vegetation × loca-

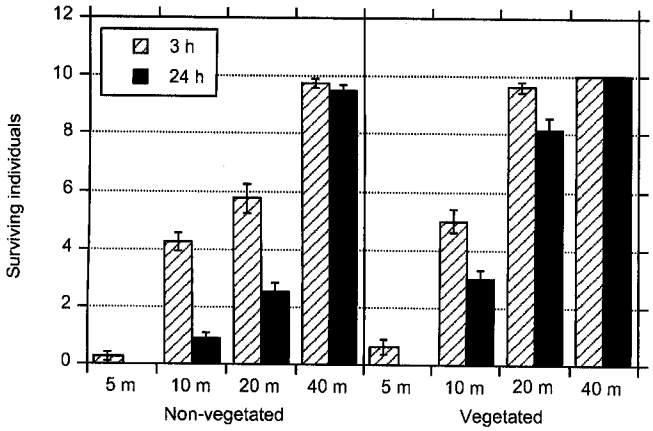

Fig. 4. Mean (± standard error, $n = 8$) survival of in situ–exposed *Chironomus tentans* in wetland mesocosms at different times after introduction of methyl parathion. Initial number of individuals was 10 per replicate.

tion (Table 2), confirming that this species was affected at a higher level in the inlet area of the nonvegetated wetland than in the inlet area of the vegetated wetland (Fig. 3a), which again highlights the positive effect of vegetation coverage on survival. A similar trend was also present in *Epitheca cynosura* and *Dromgomphus spinosus*, but the abundances of these species were too low to indicate significant differences.

In situ toxicity to C. tentans

Analysis of variance revealed a significant ($p < 0.001$) decrease in survival of in situ–exposed *C. tentans* at 3 and 24 h after exposure (Fig. 4). Furthermore, vegetation had a significant ($p < 0.001$) effect, with survival being higher in the vegetated wetland cells. This effect is mainly based on differences at the 10- and 20-m sampling stations, where survival was more than three times higher in the vegetated wetland at 24 h. Survival was generally below 10% at the 5-m station at 3 h and zero at 24 h, whereas the respective values for the 40-m station were all 95% or higher, demonstrating that a significant ($p < 0.001$) location effect also occurred.

The in situ bioassay results confirmed the invertebrate community results, in indicating a positive effect of vegetation on the spatial extent of MeP toxicity in the wetlands. Approximately 30% of the exposed larvae survived concentration levels between 40 μg/L at the 20-m station in the nonvegetated wetland and 90 μg/L at the 10-m station in the vegetated wetland during the 24-h exposure. This is in accordance with the 24-h LC50 of 58 μg/L reported for *Chironomus* sp. [33]. A 96-h EC50 of 32.3 μg/L was reported for *C. tentans* [34]. In situ exposures with chironomids have been used already for detection of insecticide toxicity in constructed wetland studies [1,2].

In summary, this study suggests that macrophyte vegetated wetlands have a strong potential to contribute to aquatic pesticide risk mitigation. A 40-m stretch of dense vegetation cover effectively reduced a target MeP concentration of about 700 μg/L to below detection limit (<0.1 μg/L). Furthermore, no effects of the pesticide were found on macroinvertebrate communities or in situ–exposed chironomids detected at 40 m from the pesticide inlet in the vegetated wetland. These results confirm the importance of vegetated buffer zones represented either as wetland areas within streams or ditches or as vegetation coverage in the streams or ditches. The conclusion can be made that the conservation and management of vegetation in small drainage channels may be an effective tool to avoid agricultural pesticide contamination of larger receiving water bodies.

Acknowledgement—This study is part of a joint research project between the U.S. Department of Agriculture (USDA)–Agricultural Research Service (Oxford, MS, USA) and the Technical University of Braunschweig, Germany. We wish to acknowledge M.M. Holland, C.T. Bryant, F. Suhling, and A. Martens. A travel grant from the USDA to R. Schulz is gratefully acknowledged. Various people provided technical assistance with the field operations. The paper benefited from reviews by J.M. Dabrowski, B. Helling, A. Thorson, and two anonymous reviewers.

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