

## LINKING INSECTICIDE CONTAMINATION AND POPULATION RESPONSE IN AN AGRICULTURAL STREAM

MATHIAS LIESS\* and RALF SCHULZ

Zoologisches Institut der Technischen Universität, Fasanenstraße 3, D-38092 Braunschweig, Germany

(Received 11 June 1998; Accepted 26 November 1998)

**Abstract**—The study aims to evaluate the impact of insecticides associated with rainfall-induced surface runoff from arable land on macroinvertebrate populations. These effects of insecticides were distinguished from the hydraulic stress also associated with surface runoff. Transient increase in discharge and insecticide contamination (maximum 6 µg/L parathion-ethyl in stream water, 302 µg/L fenvalerate in suspended particulates) was observed in a headwater stream subsequent to surface runoff from arable land. In the aquatic macroinvertebrate community, eight of the eleven abundant species disappeared, and the remaining three were reduced significantly in abundance following the insecticide-contaminated runoff. Recovery within 6 months was observed for four species and recovery within 11 months for nine species. Two species remained at a low population density for over a year. The effects of insecticides were distinguished from other parameters, such as hydraulic stress associated with surface runoff, as well. The causal connection between insecticide contamination and biological response was established by eliminating increased hydraulic stress during surface runoff using in-parallel bypass microcosms containing the dominant species *Gammarus pulex* and *Limnephilus lunatus*. The mortality of these species was similar to that of the same species in the stream. Additional microcosms, disconnected from the stream during runoff events, served as a control. Thus, the toxic potential of the runoff water is considered to be responsible for the observed effect on the macroinvertebrates. It is concluded that agricultural insecticide input may alter the dynamics of macroinvertebrate communities in streams.

**Keywords**—Pesticides    Headwater stream    Macroinvertebrates    Recovery    Microcosm

## INTRODUCTION

It is known that streams are contaminated by insecticides because of surface-water runoff from arable land [1]. Whether this contamination has a considerable effect on the aquatic community is a subject of discussion. Some authors infer such an ecological significance [2–6]. However, a firm causal connection between the contamination due to runoff and a related change of the aquatic community has not yet been established. A biological response to surface-water runoff contaminated with insecticides has so far been demonstrated only by the use of in situ bioassays [7,8]. In these investigations, the link between contamination and biotic response is limited to the test organisms in the bioassay. No link was previously established between contamination and response of the community in the stream.

This information gap apparently results from difficulties in quantifying the contamination and in distinguishing the effects of insecticide from those of other environmental parameters. The quantification is problematic and costly, because streams with an agricultural catchment area are subject to unpredictable, brief pesticide inputs following precipitation [1,9,10]. Isolating of the effects of insecticides is problematic, because the runoff-induced insecticide input is always correlated with an increase in hydraulic stress due to an increase of discharge [11]. Such short-term changes in discharge are likely to alter the community. Negative effects on macroinvertebrates can occur because of the increase of current velocity [12] and because of destabilization of substrate [13,14]. Additional parameters, such as the introduction of suspended particulates

as a result of surface runoff, might be of ecological relevance as well [15].

The aim of this study was to identify the effects of runoff-related insecticide contamination on the aquatic community in a headwater stream. Responses related to insecticides are distinguished from those related to other runoff-related parameters by different approaches: (1) An extensive quantification of relevant parameters over a long period of time together with a frequent monitoring of the macroinvertebrate community and (2) An experimental isolation of insecticide stress from other stressors (e.g., increased discharge during runoff events) with the use of bypass microcosms.

## METHODS

*Study area*

The studies were carried out in the Ohebach (52°10'N, 10°28'E), a small headwater stream (base flow: 10 L/s; peak discharge up to 150 L/s) in northern Germany. The velocity of flow comes to 0.1 and 0.2 m/s at normal discharge. The stream bed (mean ± SE of  $n = 3$ ; measurements taken in June 1994) consists of  $8.3 \pm 0.2\%$  sand,  $84.1 \pm 3.0\%$  silt, and  $7.6 \pm 0.4\%$  clay (TOC:  $3.2 \pm 0.1\%$ ). The dominant land use is agricultural, with no other sources of pollution (e.g., wastewater treatment plant, urban areas, industry). Sugar beets, winter barley, and winter wheat are the most common crops. The insecticides used during the period of investigation were parathion-ethyl, fenvalerate, and deltamethrin. The catchment area (150 m above sea level) comprises 0.9 km<sup>2</sup> of slightly sloping (2–4% gradient) fields of loess loam and clayey marl. The investigated site is located about 400 to 1,200 m downstream from the spring.

\* To whom correspondence may be addressed (m.liess@tu-bs.de).

Table 1. Mean, minimum, and maximum of the chemical and physical water parameters in the Ohebach during the study period (February 1994–April 1995)

Parameter	Minimum	Maximum
pH	8.24	8.70
Oxygen (mg/L)	9.0	10.5
Temperature (°C)	1	19.5
Ammonium (mg/L)	0.05	1.0
Nitrite (mg/L)	0.04	0.15
Nitrate (mg/L)	10	50
Orthophosphate (mg/L)	0.046	0.18
CaCO <sub>3</sub> (mg/L)	338	445
Chloride (mg/L)	20	70

### General water-quality parameters

Water-quality parameters were measured at monthly intervals and also during runoff with a runoff-triggered sampler (for method of obtaining samples, see *Sampling and Analysis of Insecticides*), with electronic meters and photometric test kits from Merck Darmstadt, Germany (Table 1).

### Discharge, turbidity, and precipitation

Precipitation (at 15-min intervals, 500 m distance to investigated site) and discharge (at 3-min intervals) data were provided by the Institute for Geography and Geocology at the Technical University of Braunschweig, Germany. Hydraulic stress was analyzed in terms of discharge and change in discharge per minute, which characterizes the drift-inducing component of stream flow better than the maximal discharge does [16]. Turbidity was measured continuously with an electronic meter.

### Sampling and analysis of insecticides

A runoff-triggered sampler provided a measurement of the maximum insecticide contamination of suspension-free water and of suspended particulates in the water by sampling the brief peak contamination levels during runoff events [10]. The duration of the main contamination peak was assumed to be approximately 1 h (detailed description of the dynamics can be found in Liess et al. [10]). In most samples, the content of suspended particulates (centrifugation at 3,000 g for 5 min.) was not enough for analysis, and only the suspension-free water was analyzed. The suspended particulates were more efficiently monitored with an SPS—a sedimentation vessel that was positioned in the stream and that was emptied every 2 weeks [17]. The analyses were performed at the Institute for Ecological Chemistry and Waste Analysis at the University of Braunschweig in Germany. Chemical data were determined once because there was a limited number of samples. The water samples were processed by solid-phase extraction with C18 columns (Bakerbond, Baker, Hannover, Germany); the insecticidal substances in the suspended particulates were extracted with acetone and were subsequently purified by column chromatography using aluminum oxide. The measurements were made with a GC/ECD (gas chromatograph NP 5990, Series II; Hewlett-Packard, Avondale, PA, USA) and were confirmed with GC/MS (negative chemical ionization, a Varian 3400 gas chromatograph [varian, Walnut Creek, CA, USA] with an HP 7673 autosampler, which was directly capillary coupled to the quadruple mass spectrometer SSQ 700 [Finnigan, Bremen, Germany]), with the following quantification limits: lindane and parathion-ethyl, 0.01 µg/L for water and 1 µg/kg (dry

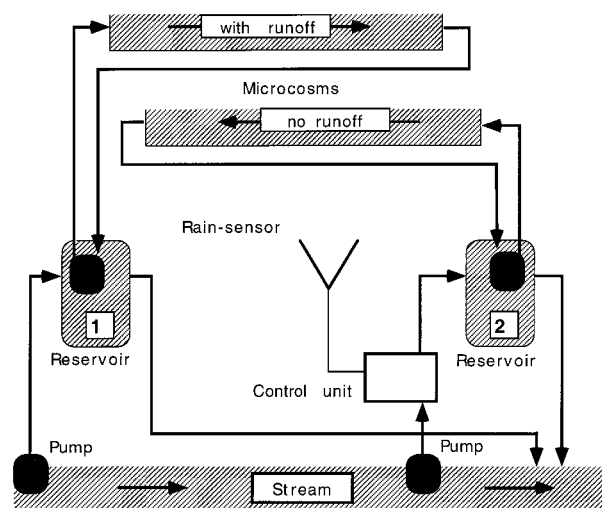


Fig. 1. Microcosms, operated in the bypass to the stream. Changes in hydraulic stress during runoff events are eliminated in both microcosms. The microcosm with runoff was continuously connected to the headwater stream and could receive the insecticide-contaminated runoff. The microcosm no runoff was cut off from the stream during precipitation and served as a control.

weight [dw]) for suspended particulates; fenvalerate and deltamethrin, 0.05 µg/L for water and 5 µg/kg (dw) for suspended particulates. These techniques are described in Liess et al. [10].

### Macroinvertebrate abundance and emergence

The invertebrates were collected on nine occasions in the time period from March 1994 to April 1995 using a Surber sampler (area: 0.062 m<sup>2</sup>; 1-mm mesh). On each occasion, eight independent samples were randomly taken over a stream length of 700 m (four samples upper reach, 400–500 m downstream from the spring; four samples lower reach, 1,100–1,200 m downstream). The sampling variation was relatively low compared with natural streams, as the stream is uniform in the longitudinal direction, with only submerged macrophytes as structural elements. About 50% of the stream bed is covered by vegetation in June, with the dominant species being *Veronica beccabunga* L., *Berula erecta* Coville, *Sparganium erectum* L., *Mentha aquatica* L., and *Glyceria fluitans* L. The macroinvertebrates were sorted out in white plastic tubs, preserved in 70% EtOH, and identified to the species level. The emergence was measured with eight emergence tents (four tents upper reach, four tents lower reach; basal surface: 1 m<sup>2</sup>, 1-mm mesh, checked weekly). Only the macroinvertebrate species present in at least two of the eight Surber samples on at least two of the nine occasions were included in the subsequent analysis. All other species (e.g., chironomids, leeches, and beetles) were excluded from the analyses because of their scarcity. The statistical analysis of the reductions of macroinvertebrate population densities was made by means of a two-way (time, location) analysis of variance (ANOVA). Scheffé's *F* test was used to detect significant differences among means.

### Microcosms in bypass to the stream

The toxic potential of surface runoff was assessed in two artificial channels supplied with water from the headwater stream described above. One of these channels was continuously provided with water from the headwater stream by means of electrical pumps (Fig. 1, with runoff). The other channel was cut off from the stream water supply during precipitation,

Table 2. Insecticide contamination in the water (1-h peak concentration) and the suspended particulates (14-d composite sample), discharge, and turbidity during runoff events in the Ohebach<sup>a</sup>

Date (1994)	No. of event	Parathion-ethyl <sup>b</sup>		Discharge increase (%/min)	Turbidity (FTU)
		Water (µg/L)	Suspended particulate (µg/kg, dry wt)		
March 1	1	—	2.2 (7.3.)	2.29	294
March 17	2	—	ND (29.3.)	2.20	331
April 13	3	—	1.0 (17.4.)	3.47	522
April 25	4	0.04	ND (28.4.)	4.30	643
Mai 19	5	6.0	50.8 (26.5)	3.05	273
Mai 25	6	0.9	50.8 (26.5)	2.05	127
June 8	7	0.2	19.4 (9.6.)	3.45	508
<sup>c</sup>			8.2 (16.6.)		
August 13	8	ND	2.2 (18.8.)	6.44	581
August 18	9	ND	2.2 (18.8.)	6.66	612
August 24	10	ND	20.0 (1.9.)	4.17	582
October 3	11	ND	6.0 (15.9.)	0.65	494

<sup>a</sup> — = not analyzed; ND = not detected; dry wt = dry weight; FTU = formazine turbidity unit.

<sup>b</sup> Suspended particulates consist of a composite sample collected over 2 weeks. The date in brackets indicates the last day of the 2-week interval. During event 7, the insecticide fenvalerate was detected in suspended particulates with a concentration of 71 µg/kg, suspended particulates were obtained with the runoff-triggered sampler. The 1-h peak concentrations were 62 µg/kg for parathion-ethyl and 302 µg/kg for fenvalerate.

<sup>c</sup> The values in this line do not correspond directly to a surface-water runoff. As no input of insecticides occurs in that sampling interval, the decrease in the contamination of suspended particulates could be monitored.

and its internal circulation was maintained without allowing runoff water to enter (Fig. 1, no runoff). One hour after the rainfall had stopped, the communication between this channel and the stream was restored. Three plastic cages were hung in each channel, with water flowing through them at 0.15 m/s. The front and back walls of the cages were made of gauze and were covered with net curtain material (1.5-mm mesh) in order to retain emerged Trichoptera. Each cage had a floor area of 0.12 m<sup>2</sup> and contained 75 larvae of *Limnephilus lunatus* Curtis (Trichoptera) and 25 individuals of *Gammarus pulex* L. (Amphipoda), along with 100 g brook sediment and nine 20-cm-long *B. erecta* plants to serve as case-building material and food. The densities of the macroinvertebrates and plants in the microcosms corresponded to the natural densities. About every 10 d, larvae and emerged individuals were counted. In order to test the change of water quality when disconnected from the stream, the supply of stream water to the artificial channel "no runoff" was switched off for 72 h prior to the experiment. The internal reservoir was large enough (200 L) that the water quality did not change, and no damage to the two macroinvertebrate species was observed. During runoff, the pump was switched off for a maximum of 28 h. The statistical analysis of the population density reduction of the macroinvertebrates in the artificial channels with runoff and with no runoff was performed by means of a chi-square, Fisher's exact test; the data from the three cages in each artificial channel were combined.

## RESULTS

### Stress factors related to the runoff events

Transient high-peak insecticide loads were monitored with the runoff-triggered sampler. The contamination of the stream and the discharge and turbidity during runoff events are summarized in Table 2. The values for discharge and turbidity were used as indicators for hydraulic stress.

The insecticides parathion-ethyl and fenvalerate were applied in the catchment of the Ohebach in 1994. The first ap-

plication of parathion-ethyl took place on May 18, 1994, the subsequent runoff event on May 19, 1994. Prior to this event, the parathion-ethyl concentration did not exceed 0.04 µg/L in the water or 2.2 µg/kg in the suspended particulates. Fenvalerate was first applied on June 6, 1994, and the next runoff event occurred on June 8, 1994. Prior to this event, the fenvalerate concentration in the water and in the suspended particulates was below the detection limit.

The insecticide lindane (not applied) was detected in the suspended particulates of the stream with a mean concentration of 1.5 µg/kg dw. During runoff events, no increase in the level of contamination could be observed. The insecticide deltamethrin was not detected.

Of all runoff events, only event numbers 5, 6, and 7 showed an increased insecticide contamination in the water and the suspended particulates that was considerably more than one order of magnitude above the background concentration (Table 2). These events were graded as high contamination. All other events were graded as low contamination. Events 1, 2, and 3 were graded as low, since they occurred prior to the period of insecticide application and since the suspended particulates did not show elevated concentrations during that time.

### Effects of runoff on the macroinvertebrate community in the stream

Figure 2 shows the number of species in the headwater stream, the discharge, and the runoff events over the course of time. Shortly after the runoff events graded as high contamination, the number of species decreased significantly (AN-OVA, Scheffé's *F* test, *p* < 0.0001). In contrast, no correlation was found between the hydraulic stress (discharge, change of discharge) or the turbidity and the reduction of species number. The runoff events graded as low contamination were associated with similar or, in some cases, even higher values of the hydraulic stress components, when compared with high-contamination runoff events (see Fig. 2 and Table 2).

The abundance dynamics of the different species and the

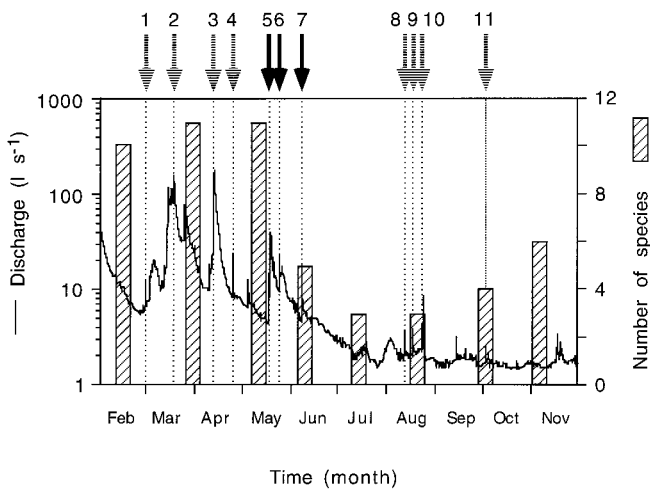


Fig. 2. Number of species and discharge over the course of time. Arrows indicate runoff events: black, with high insecticide contamination; shaded, with low insecticide contamination. Exact values of insecticide contamination, discharge, and turbidity during runoff events are shown in Table 2.

occurrence of the runoff events with high insecticide contamination (arrows) are shown in Figure 3. The population density of all species decreased significantly shortly after the occurrence of the three highly contaminated runoff events. None of the merolimnic species emerged in the investigated period. Eight of the eleven abundant species disappeared, and the remaining three species were reduced significantly in abundance following the insecticide-contaminated runoff. Recovery within 6 months was observed for four species (Fig. 3A) and within 11 months for nine species (Fig. 3A and B). Two species remained at a low population density for about a year (Fig. 3C). No correlation between the abundance of species and the intensity of the hydraulic stress was found for any of the species.

#### Effects of runoff on two species in bypass microcosms

Both investigated species (*L. lunatus* and *G. pulex*) showed a higher decrease of survival in microcosms with runoff than in the no-runoff microcosms. This applies to *L. lunatus* particularly during the period of highly contaminated runoff events 5 and 6 and also to a lesser extent during the low-contamination runoff events 3 and 4. As a result, the population density in microcosms with runoff was significantly lower than in those with no runoff from the end of April to the end of the test period (chi-square,  $p < 0.0001$ , Fig. 4A). None of the larvae emerged throughout the whole test period.

With the species *G. pulex*, a reduction of survival in the treatment with runoff compared with the treatment no runoff also occurred during the period of highly contaminated runoff events 5 and 6 (Fig. 4B). Thus, the population density in microcosms with runoff was also significantly lower than in those with no runoff in the samples taken at the beginning of June (chi-square,  $p < 0.022$ ). In contrast to *L. lunatus*, this difference between the two sets of microcosms did not remain significant during the remainder of the test period. Furthermore, runoff events 3 and 4 with low contamination did not reduce the survival of this species in the microcosms with runoff.

The effects of the runoff events on the microcosms with runoff are comparable to those in the stream. The strongest

effects—the near elimination of *L. lunatus* in the microcosm—were observed subsequent to runoff events 5 and 6 in the microcosms and events 5, 6, and 7 in the stream. To a lesser extent, a similar situation could be observed with *G. pulex*. In the microcosms with runoff and in the stream, the density of this species was reduced to one-half during runoff events 5, 6, and 7.

Since the survival in the microcosms was monitored more frequently, it is possible to differentiate the effects of these three particular events. After runoff events 5 and 6, which were contaminated with parathion-ethyl, nearly all individuals of *L. lunatus* in the microcosms with runoff had already died, whereas the numbers of *G. pulex* were significantly reduced. There was no appreciable further decrease in survival of *G. pulex* during runoff event 7, which was contaminated with fenvalerate (Table 2).

## DISCUSSION

### Analysis of parameters responsible for the observed effects

All species of macroinvertebrates found in the headwater stream showed a significant population reduction during the highly contaminated runoff events with an insecticide concentration well above the background level (multiyear monitoring, Liess et al. [10]). In contrast, no negative effect on the macroinvertebrates was detected in the stream during the events with low contamination. The results strongly suggest that the insecticide contamination of runoff events 5, 6, and 7 was responsible for the observed population reduction. All the other parameters monitored appeared to be of minor importance.

Hydraulic stress apparently plays a minor role for the population reduction during the highly contaminated runoff events, given that during runoff events with a low insecticide concentration, the discharge, the change of discharge, and the load of suspended particulates were similar or even higher (Table 2 and Fig. 2). In addition, the results from the microcosms support the idea that hydraulic stress is of minor importance for the investigated macroinvertebrates. The abundance of both species was also significantly reduced in the microcosms with insecticide contamination (with runoff) but without hydraulic stress during the highly contaminated runoff events 5, 6, and 7. The decrease in abundance of both species is similar in the microcosms (with runoff) and in the headwater stream. *Limnephilus lunatus* nearly disappeared in the microcosm experiment and in the headwater stream because of the highly contaminated runoff, whereas the abundance of *G. pulex* was reduced to about one-half in the microcosms and in the headwater stream (Figs. 3 and 4). The microcosms with no runoff served as a control; here, neither of the two investigated species showed an increase in mortality during the highly contaminated runoff events.

Nevertheless, there is an indication that suspended particulates can affect *L. lunatus* but not *G. pulex*. During runoff events 3 and 4, a significant reduction in the larvae of this species was observed in the microcosms with runoff (Fig. 4A). Both events were correlated with a high turbidity (Table 2). No such population decline was observed in the stream. This difference might be due to longer intervals of sampling in the stream. It is likely that the effects of events 3 and 4 were obscured by other processes, such as migration.

Ammonium, nitrite, and nitrate are not likely to play substantial roles in governing the population dynamics of the investigated species in the Ohebach. First, the observed con-

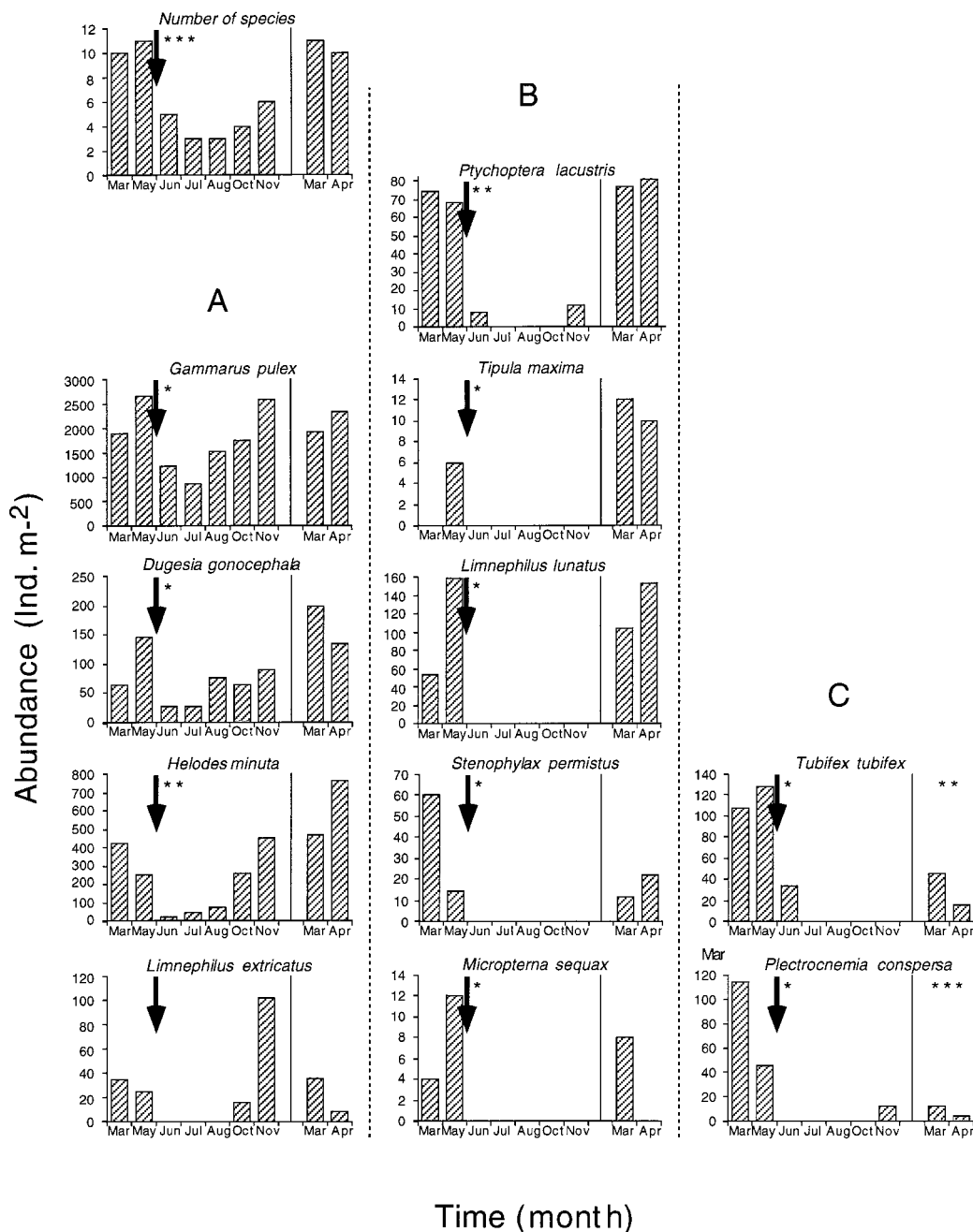


Fig. 3. Abundance of various macroinvertebrate species over the course of the season. The arrows indicate the period of the runoff events with high insecticide contamination (5, 6, and 7; see Table 2). The significance levels refer to the reduction of abundance before and after the insecticide input and to the differences between the spring abundances in the 2 years (analysis of variance, Scheffé's *F* test,  $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*)). Species grouped according to observed recovery: (A) 6 months. (B) 11 months. (C) no recovery within 11 months.

centrations (Table 1) are far below concentrations that are found to be toxic for closely related species [18–20]. Second, hydrophilic ions (like  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$  originating from arable land) are at a minimum concentration in the stream during the surface runoff; the maximum concentration is reached several hours to a few days later, because they are mainly introduced after passage through the soil [21]. This has also been previously shown for the investigated site, the Ohebach [22]. As the microcosms without runoff were reconnected to the stream water supply a few hours after the actual runoff, they received the elevated concentrations of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$  after runoff events. These concentrations apparently do not affect the investigated species, in view of the finding that

no runoff-related decrease of individuals occurred in the microcosms without runoff (Fig. 4).

Deposition of insecticides due to spray drift was not monitored within this study, but the pattern of the biological response in the microcosms without runoff gives no evidence that major importance is attributable to this pathway of contamination. These microcosms, disconnected from the water supply only during rainfall, continued to receive stream water during spraying of insecticides. No increased mortality of either test species was observed in the microcosm without runoff during the application of insecticides (May 18 and June 6, 1994).

Considering these aspects, we conclude that the insecticide

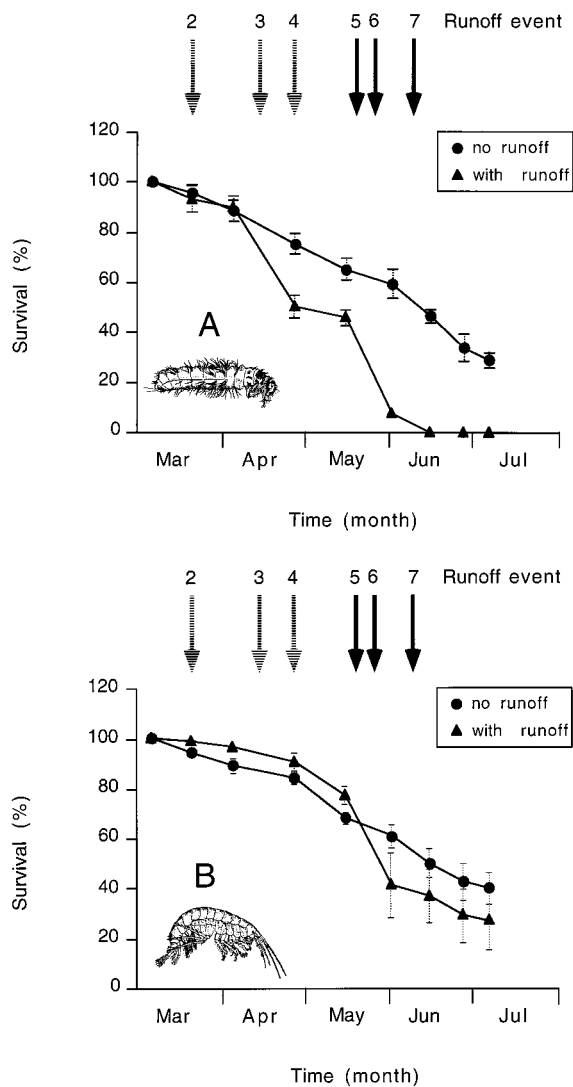


Fig. 4. Mean survival rate ( $\pm 1$  SE) of *Limnephilus lunatus* (A) and *Gammarus pulex* (B) in microcosms with and without surface runoff. Arrows indicate runoff events: black, with high insecticide contamination; grey, with low insecticide contamination. Insecticide contamination during runoff events is shown in Table 2.

contamination of the runoff is most likely responsible for the observed population decrease in the stream and in the microcosms.

#### Concentration–response relationship of insecticides in the field

The results of this investigation strongly suggest that the parathion–ethyl contamination is responsible for the observed biological effects. The distinct toxicological importance of parathion–ethyl–contaminated runoff events (5, 6) compared with the fenvalerate–contaminated runoff event (7) was established because of the high time resolution of abundance measurement in the microcosms. The main abundance reduction of both species occurred during runoff events 5 and 6. As fenvalerate was measured only in association with suspended particulates, a reduced bioavailability could be assumed in the investigated case. However, this result does not necessarily imply a higher toxicity of parathion–ethyl under field conditions, since the first contamination of a previously uncontam-

inated community elicits the strongest biological response [23].

The strongest effects—the near elimination of *L. lunatus* in the microcosm—were observed subsequent to runoff events 5 and 6. In the stream, too, comparable effects on *L. lunatus* were observed following runoff events 5, 6, and 7. A similar but less severe situation was observed in the case of *G. pulex*. In the microcosms with runoff and in the stream, the density of this species was reduced to one-half during the runoff events.

A noteworthy observation resulting from this study is that under field and microcosm conditions, the reactions of both species were stronger than the response predicted using laboratory toxicity data. It is assumed that the parathion–ethyl concentration of  $6 \mu\text{g/L}$  measured in the water for event 5 (Table 2) lasted only for a short time: approximately 1 h [10]. For *L. lunatus*, nearly eliminated in the microcosm and in the stream, this short-term peak concentration is lower by a factor of 10 than the LC50 of  $64 \mu\text{g/L}$  for a 1-h exposure followed by a 24-h observation period [24]. The results obtained with *G. pulex* confirm these findings; for *G. pulex*, which was reduced by one-half in the microcosm and in the stream, this short-term peak concentration is a factor of approx. 500 lower than the LC50 of  $3,500 \mu\text{g/L}$  for 1 h of exposure followed by a 24-h observation period [24]. According to the equilibrium partitioning concept, the measured contamination of suspended particulates should not be of significant toxicity. The maximum of  $50.8 \mu\text{g/kg}$  for the time interval between the May 19 and 20, 1994, should result in a contamination of the water of approx.  $3 \text{ ng/L}$  [25], which would not significantly contribute to the short-term contamination. Hence, in view of the concentrations measured in the field, it is not possible to fully explain the observed mortality reaction of both species.

There are several possible explanations for the difference between the concentration–response relationships in the field and in the laboratory. It might be that the duration of contamination was longer than assessed or that the elevated contamination of suspended particles (Table 2) increased the overall exposure of the organisms more than was calculated above. A higher sensitivity of the investigated species under field conditions as opposed to laboratory tests could be another possibility. Similar findings have already been described: 100% mortality of caged *G. pulex*, following a peak concentration of  $27 \mu\text{g/L}$  carbofuran, was observed; this exceeds the 24-h LC50 of  $21 \mu\text{g/L}$  only for 3 to 5 h [8]. Also, other authors [7] expected differences in measured and real exposure concentrations to be a reason for higher mortalities in situ bioassays than was predicted by laboratory data.

#### Recovery from insecticide contamination

The results of this study show that 1 year after the observed runoff event, which was contaminated with insecticides, two out of the eleven investigated species had not yet fully recovered. Given that no emergence of the merolimnic insects was observed in this stream, it seems likely that for many species, recovery was due to recolonization from less affected sites. In the present example, a small headwater stream surrounded by meadows is located 500 m away from the study site. No contamination with insecticides was detected in this stream during several years. Hence, a recolonization from this stream could be possible.

It is obvious, however, that some species are not necessarily dependent on less contaminated refugial streams. Out of the

11 investigated species, three did not fully disappear after the runoff event (Fig. 2). So it can be expected that their recovery is partly due to reproduction of the autochthonous population. Considering this ability, the following species could be classified as relatively insensitive to insecticide contamination: *G. pulex*, *Dugesia gonocephala*, and *Helodes minuta*.

The other eight investigated species disappeared temporarily from June on, or during July at the latest, from the investigated stream section after the contaminated runoff event. Out of these eight species, the seven merolimnic insects showed no emergence in the investigated stream section, and all occurred in other streams of the same area for long periods in the same year [26]: *Tubifex tubifex*, *Ptychoptera lacustris*, and *Tipula maxima* throughout the whole year; *L. lunatus* until the end of September; *Plectrocnemia conspersa* until the end of July; and *Limnephilus extricatus*, *Stenophylax permistus*, and *Micropterna sequax* until the end of June. Therefore, these eight species are classified as sensitive to insecticide contamination.

#### *Missing link between hydraulic stress and macroinvertebrate community*

Some authors have found negative effects of hydraulic stress on macroinvertebrate communities. An increase of the current velocity can reduce larval size [12]. Substrate displacement reduces macroinvertebrate abundance [14] and reduces emergence of Trichoptera [13]. In view of these findings, some negative effects of the hydrodynamic stress on the macroinvertebrates investigated in this study might have been expected. We argue that the reason they were not observed in the stream studied here is that the community structure is already adapted to this type of stress because of its frequent occurrence in runoff-affected streams.

In the Ohebach, more than 11 runoff events involving subsequent hydraulic stress and transport of suspended particulates occurred in the year of investigation. More than 10 runoff events per year involving increased hydraulic stress were also measured in previous years [10]. Hence, agricultural headwater streams are subject to an average of about one runoff event per month. As the recovery of disturbed species by recolonization takes a few months [23,27], the species found in this headwater stream must be able to stand the approximately monthly recurrence of increased hydraulic stress. This implies that the macroinvertebrate community is adapted to hydraulic stress. In such a situation, it is thus comprehensible that no response of macroinvertebrates to the hydraulic component of runoff events was measured.

#### *Applicability to other headwater streams with agricultural catchment*

The authors conclude that the present results do apply to other small headwater streams. With respect to catchment morphology and the meteorological situation, the insecticide contamination measured here can be considered to be average. The amount and frequency of precipitation during the years of the study correspond to the average of the last 9 years [10]. Other authors have observed a similar frequency of insecticide input [9,28]. However, the results of the present study are not transferable to streams with a bigger catchment area. As a result of dilution effects, sedimentation, and decay of insecticides along the longitudinal gradient, the contamination is expected to be lower in streams with larger catchment areas [28–30].

## CONCLUSIONS

We demonstrated that agricultural insecticide input is altering the dynamics of the macroinvertebrate community in the investigated stream. If these findings are validated for a variety of streams, the future ecotoxicological assessment of insecticides should not only be based on laboratory and mesocosm investigations but should also give more consideration to the situation in the field.

*Acknowledgement*—This study is part of the Special Collaborative Program 179 (Water and Matter Dynamics in Agroecosystems) and was supported by the German Society for the Advancement of Scientific Research (DFG). We are grateful to K. Hasenpusch, Federal Research Center for Agriculture for providing discharge data and to P. Matthiessen and four anonymous reviewers for their knowledgeable comments on the manuscript.

## REFERENCES

1. Wauchope RD. 1978. The pesticide content of surface water draining from agricultural fields—A review. *J Environ Qual* 7:459–472.
2. Aufseß G, et al. 1989. Untersuchungen zum Austrag von Pflanzenschutzmitteln und Nährstoffen aus Rebflächen des Moseltals. In Deutscher Verband für Wasserwirtschaft und Kulturbau e.V., *Stoffbelastungen der Fließgewässerbioptoe* Parey, Berlin, Germany, pp 1–78.
3. Heckman CW. 1981. Long-term effects of intensive pesticide applications on the aquatic community in orchard ditches near Hamburg, Germany. *Arch Environ Contam Toxicol* 10:393–426.
4. Lenat DR, Crawford JK. 1994. Effects of land use on water quality and aquatic biota of three North Carolina piedmont streams. *Hydrobiologia* 294:185–199.
5. Sallenaive RM, Day KE. 1991. Secondary production of benthic stream invertebrates in agricultural watersheds with different land management practices. *Chemosphere* 23:57–76.
6. Tada M, Shiraishi H. 1994. Changes in abundance of benthic macroinvertebrates in a pesticide-contaminated river. *Jpn J Limnol* 55:159–164.
7. Baughman DS, Moore DW, Scott GI. 1989. A comparison and evaluation of field and laboratory toxicity tests with fenvalerate on an estuarine crustacean. *Environ Toxicol Chem* 8:417–429.
8. Matthiessen P, Sheahan D, Harrison R, Kirby M, Rycroft R, Turnbull A, Volkner C, Williams R. 1995. Use of a *Gammarus pulex* bioassay to measure the effects of transient carbofuran runoff from farmland. *Ecotoxicol Environ Saf* 30:111–119.
9. Kreuger J. 1995. Monitoring of pesticides in subsurface and surface water within an agricultural catchment in southern Sweden. *Pesticide Movement to Water. Br Crop Prot Counc Monogr* 62: 81–86.
10. Liess M, Schulz R, Rother B, Kreuzig R. 1999. Quantification of insecticide contamination in agricultural headwater streams. *Water Res* 33:239–247.
11. Gomme JW, Shurvell S, Hennings SM, Clark L. 1991. Hydrology of pesticides in a chalk catchment—Surface waters. *J Inst Water Environ Manage* 5:546–553.
12. Statzner B. 1981. The relation between “hydraulic stress” and microdistribution of benthic macroinvertebrates in a lowland running water system, the Schierenseebrooks (North Germany). *Arch Hydrobiol* 91:192–218.
13. Cobb DG, Flannagan JF. 1990. Trichoptera and substrate stability in the Ochre River, Manitoba. *Hydrobiologia* 206:29–38.
14. Peckarsky BL. 1991. Habitat selection by stream dwelling predatory stoneflies. *Can J Fish Aquat Sci* 48:1069–1076.
15. Cooper CM, Lipe WM. 1992. Water quality and agriculture: Mississippi experiences. *J Soil Water Conserv* 47:220–223.
16. Poff NL, Ward JV. 1991. Drift responses of benthic invertebrates to experimental streamflow variation in a hydrologically stable stream. *Can J Fish Aquat Sci* 48:1926–1936.
17. Liess M, Schulz R, Neumann M. 1996. A method for monitoring pesticides bound to suspended particles in small streams. *Chemosphere* 32:1963–1969.
18. Jensen FB. 1996. Uptake, elimination and effects of nitrite and nitrate in freshwater crayfish (*Astacus astacus*). *Aquat Toxicol* 34:95–104.
19. Neumann D, Volpers M, Raschke I, Grauel U, Cichors F. 1994.

- What is known on natural limiting factors for macrozoobenthos species in freshwaters? In Hill IR, Heimbach F, Leeuwangh P, Matthiessen P, eds, *Freshwater Field Tests for Hazard Assessment of Chemicals*. Lewis, Boca Raton, FL, USA, pp 525–531.
20. Schulz R. 1997. *Aquatische Ökotoxikologie von Insektiziden—Auswirkungen diffuser Insektizideinträge aus der Landwirtschaft auf Fließgewässer-Lebensgemeinschaften*. Ecomed Verlag, Landsberg, Germany.
  21. Kohonen T. 1982. *Influence of Sampling Frequency on the Estimates of Runoff Water Quality*. Water Research Institute, Helsinki, Finland.
  22. Walther W. 1980. Prozess des Stoffabtrages und der Stoffauswaschung während und nach Starkregen in ackerbaulich genutzten Gebieten—2. Bericht: Stoffauswaschung. *Z Kulturtech Flurbereinig* 21:65–74.
  23. Wallace JB, Huryn AD, Lugthart GJ. 1991. Colonization of a headwater stream during three years of seasonal insecticidal applications. *Hydrobiologia* 211:65–76.
  24. Liess M. 1994. Pesticide impact on macroinvertebrate communities of running waters in agricultural ecosystems. *Proc Int Assoc Theor Appl Limnol* 25:2060–2062.
  25. Hornsby AG, Wauchope RD, Herner AE. 1995. *Pesticide Properties in the Environment*. Springer Verlag, New York, NY, USA.
  26. Sta WA. 1994. *Gewässergütebericht: Ergänzungen 1994*. Staatliches Amt für Wasser und Abfall Braunschweig, Braunschweig, Germany.
  27. Heckman CW. 1983. The recovery of the biotic community in a lotic freshwater habitat after extensive destruction by chlorine. *Int Rev Gesamten Hydrobiol* 68:207–226.
  28. Williams RJ, Brooke D, Matthiesen P, Mills M, Turnbull A, Harrison RM. 1995. Pesticide transport to surface waters within an agricultural catchment. *J Inst Water Environ Manage* 9:72–81.
  29. Sibley PK, Kaushik KN, Kreutzweiser DP. 1991. Impact of a pulse application of permethrin on the macroinvertebrate community of a headwater stream. *Environ Pollut* 70:35–55.
  30. Iwakuma T, Shiraishi H, Nohara S, Takamura K. 1993. Runoff properties and change in concentrations of agricultural pesticides in a river system during a rice cultivation period. *Chemosphere* 27:677–691.