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Runoff-Related Endosulfan Contamination and Aquatic Macroinvertebrate Response in Rural Basins Near Buenos Aires, Argentina

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Abstract. Information in the open literature about the fate and effects of pesticides in small streams from agricultural areas of Argentina is very rare. The objective of the present work was to study the pesticide contamination and potential biological effects in basins that have undergone intense agricultural activity, mainly related to the cultivation of soybeans. Three streams (Maguire, Helves, and Horqueta) with a low-flow discharge (0.1 and 0.2 m^3/s) in March close to the city of Arrecifes were studied during the period of maximum insecticide application, between February and April 2001. Various sampling devices were installed to trap suspended particles, runoff, and floodwater plus sediment throughout the study period. The suspended-particle samples were analyzed for the insecticides endosulfan (END), chlorpyrifos, and cypermethrin. Water chemistry and the macroinvertebrate communities were assessed on four occasions and the organismic drift was measured continuously. Following a 184-mm rainfall on March 1, 2001, β-endosulfan concentrations up to 318 and 43 μg/kg were measured from suspended-particle samples from Horqueta and Helves, respectively. No END contamination was detected in Maguire. Chlorpyrifos and cypermethrin were not detected in any of the streams. A significant decrease in the average macroinvertebrate species density was observed in Horqueta (from 12.8 ± 0.5 to 9 ± 0.7 species; ANOVA, $p < 0.05$) and Helves (from 10.8 ± 1.7 to 3.3 ± 1.3 species; $p < 0.001$) following the same rainfall event at the beginning of March, while the species density in Maguire remained constant at 7.9 ± 0.3 species. The runoff primarily reduced species abundances of Odonata and Ephemeroptera significantly ($p < 0.01$) in Horqueta and Helves but not in Maguire. A greater drift of Smicridae (Trichoptera) and Ephemeroptera occurred in Helves and Horqueta during this runoff event, while no changes in the macroinvertebrate drift were detectable in Maguire. This study highlights the potential pesticide effects on macroinvertebrate communities in Argentinian rural streams. It is suggested that a small wetland area formed by Maguire between the agriculturally used catchment and the sampling site contributes to the absence of contamination and effects at this site.

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Soybean production in the province of Buenos Aires has increased notably, from 2.8 million tons in 1991 to 3.9 million tons in 1998 (SAGPyA 2002). The rapid implementation of new technological developments, such as the utilization of a transgenic soybean resistant to glyphosate and the direct seed modality, has contributed to elaboration of soybean and related products (oil, flours, pellets) into the main Argentine export, earning about $5 billion U.S. (Clarín 2002). Increased soybean production was closely followed by increased pesticide consumption, which has grown from 39 million to 124 million kg between 1991 and 1997 (Pengue 2000). The study area for this investigation is located in the main region of soybean production in the province of Buenos Aires. Most of the insecticides currently in use are pyrethroids, which are recently preferred to organochlorine or organophosphate pesticides because of their comparatively low toxicity to humans and their shorter half-lives in the field. Fifty percent of the total amount of insecticides used in the soybean area of Buenos Aires is accounted for by the pyrethroid cypermethrin, followed by the organophosphate chlorpyrifos, the pyrethroid deltamethrin, and the organochlorine endosulfan. Pesticides are freely sold to farmers in rural areas, and management practices performed by the farmers are difficult to supervise.

The comparatively large size of the fields makes airplane fumigation very common within the area, which might cause direct pesticide input into surface waters (Ernst *et al.* 1991). However, the study region is also characterized by short and heavy rainfall events in summer with 1- or 2-week dry periods in between. Thus, rainfall-induced surface runoff from agricultural fields may be another important nonpoint route of pesticides into surface waters (Wauchope 1978; Willis and McDowell 1982; Schulz *et al.* 1998).

It has been reported in various studies that pesticides in surface runoff are often associated with suspended particles (House *et al.* 1992; Mian and Mulla 1992), and even single runoff events have been found to transport large amounts of insecticides into surface waters (Domagalski *et al.* 1997;

Schulz 2001; Dabrowski *et al.* 2002). However, a direct link between runoff-related insecticide input into surface waters and the resulting biological effects have rarely been established (Schulz and Liess 1999). Recent studies have attempted to link the endosulfan contamination in the Namoi River of Eastern Australia with impacted macroinvertebrate communities (Leonard *et al.* 2000, 2001).

Basic information on the natural species composition and density of invertebrate communities of the Pampasic streams remains largely unreported (Marchese and De Drago 1992). The present contribution represents one of the first approaches to provide a description of the relationship between insecticide contamination and aquatic macroinvertebrate community dynamics in Pampasic streams of Argentina associated with the Rio de La Plata catchment area, as intense soybean production. However, the presence of chlorinated pesticides in the Rio de La Plata estuary has been clearly documented (Colombo *et al.* 1995). The study focused on runoff-related insecticide contamination and the link to potential effects on macroinvertebrate communities in three streams about 175 km east of Buenos Aires, Argentina. During the sampling period, two main periods with rainfall of more than 100 mm/day occurred, and it was observed that even rainfall of 26 mm/day resulted in edge-of-field runoff events in all investigated streams.

Materials and Methods

Study Sites

Three streams belonging to the Rio de La Plata catchment were chosen (Maguire, Helves, and Horqueta), in an area in which soybean is the main crop, close to the city of Arrecifes (34°03′S; 60°18′W), in the Buenos Aires province. Each stream was sampled at its intersection with Route No. 8, 150 to 196 km from Buenos Aires. Sampling was performed from February to April 2001, the period during which soybean is treated with insecticides. Horqueta and Maguire developed a floodplain containing abundant emergent and submersed macrophytes. Helves, on the other hand, has steep slopes and lacks a floodplain (Table 1). Across Horqueta, a dam has been built for recreational purposes 300 m upstream of the sampling site. Maguire widens to form a small wetland (0.055 ha) colonized by dense stands of emergent macrophytes, mainly bulrush (*Schoenoplectus californicus*), 300 m upstream of the sampling site. In Horqueta and Helves, potential agricultural edge-of-field runoff input sites as indicated by erosion rills are present directly upstream of the sampling site, while in Maguire the wetland is situated between the potential runoff input sites and the sampling site.

Pesticide Sampling

Passive sampling devices were installed at each site to sample stream water during flood-induced peak water level. Bottles (0.5 L) with two holes 5 mm in diameter in the lid were mounted on sticks, with the opening about 5 or 15 cm higher than the water surface during low water periods (Schulz *et al.* 2001). After floods produced by heavy rain, the bottles were recovered and the suspended particles were used for chemical analysis.

The sediments suspended in the water phase of the stream were accumulated continuously by a suspended-particle sampler (Liess *et al.* 1996), from which they were collected and analyzed between February and April 2001 at 10-day intervals. The suspended-particle sampler, which consists of a 3-L bottle firmly attached to sticks, was installed in the bottom of each stream with an inlet (opening, 10 × 3 mm) and an outlet pipe. Suspended particles washed into the streams via edge-of-field runoff were sampled with specific runoff samplers (Schulz *et al.* 1998), consisting of 3-L containers placed in erosion rills between agricultural fields and the streams.

Analytical Procedures

Suspended-particle samples were extracted twice with 50 ml methanol in an ultrasonic bath for 30 min and then passed through C18 columns (Bakerbond; solid-phase extraction) and frozen until analysis. The pesticide analysis was performed after elution of the C18 columns with 2 ml hexane followed by 2 ml dichloromethane. The sample extracts were injected into a gas chromatograph fitted with standard electron capture and flame photometric detectors. Detection limits were 2 μg/kg dry weight for endosulfan and chlorpyrifos and 5 μg/kg for cypermethrin; the details of the analytical procedures are outlined by Schulz (2001). All results given for END (endosulfan) refer to the β-isomer, as the α-isomer and END-sulfate were not detected in any of the samples.

Surface stream water samples were taken with bottles filled by hand for chemical analysis. Nitrate, ammonium, and soluble reactive phosphorus concentrations in the stream water were determined following standard methods (APHA 1985).

Macroinvertebrate Dynamics

Four replicate samples were taken, on February 5 and 26, March 19, and April 3, 2001, in vegetated areas of each stream with a surber sampler (0.125 m^2; mesh size, 1 mm). All macroinvertebrates captured within the sampler were counted, preserved, and identified.

Macroinvertebrate Drift

Downstream drift of macroinvertebrates was sampled by means of cylindrical plastic nets (20-cm diameter, 1 m long, 1-mm mesh size) installed in each stream at the bottom in duplicate. All macroinvertebrates that were captured within 9-day periods were counted, preserved, and identified. During the sampling period, five and six samples were collected from Maguire and Horqueta, respectively. Only three drift samples were taken in Helves during a flood period when the driftnet could not be recovered.

Data Analysis

Differences in species density and invertebrate abundances between sampling dates were tested using analysis of variance (ANOVA) followed by Fisher's protected least-significant-difference test. Data were transformed using $\ln(x + 1)$ prior to statistical analysis to satisfy the assumptions of the statistical tests.

Results

Pesticides in Suspended Particles

Precipitation through the study was mainly concentrated in two rainfall periods lasting from February 9 to February 12 (134 mm)

Table 1. Characteristic parameters of the catchment areas and the study streams: Means (±SE; $n = 8$) for physicochemical water quality parameters

	Maguire	Horqueta	Helves
Arable land in catchment (ha)	390	400	380
Mean slope (%)	2	3	1.5
Minimal buffer strip width (m)	8	10	12
Average streambed width (m)	4	3.5	2
Average water depth (m)	0.6	0.4	0.4
Low-flow discharge in March (m^3/s)	0.2	0.1	0.1
In-stream macrophyte coverage (%)	2	4	2
pH	8.1 ± 0.3	8.2 ± 0.4	8.2 ± 0.3
Oxygen content (mg/L)	7.6 ± 2.0	7.4 ± 2.5	9.3 ± 2.7
Specific conductivity (μS/cm)	943 ± 169	870 ± 113	861 ± 65
Soluble reactive phosphate SRP (mg/L)	0.13 ± 0.09	0.15 ± 0.14	0.14 ± 0.04
NNH_4^+ (mg/L)	0.03 ± 0.01	0.03 ± 0.03	0.02 ± 0.01
NNO_3 (mg/L)	1.4 ± 1.2	1.1 ± 0.8	3.6 ± 4.0

and from March 1 to March 6 (229 mm) (Fig. 3). END was found in suspended particles of Horqueta and Helves after the rainfall event of 184 mm/day on March 1. The amounts present in these samples were 30 and 43 μg/kg, respectively, while a suspended-particle sample of edge-of-field runoff into Helves contained 10 μg/kg. In Horqueta, a suspended-particle sample taken during flood conditions contained 318 μg/kg, while in Maguire no END was detected (peak samples in Table 2). On the following sampling date, 2 weeks later, no END was found in any of the studied streams (postpeak sampling in Table 2).

An additional analysis of bottom sediments taken on February 26 in the wetland of Maguire revealed END concentrations of 109 μg/kg, while bottom sediments in Helves and Horqueta contained 21 and 17 μg/kg END. Chlorpyrifos and cypermethrin were not detectable in any sediment or suspended-particle sample (for detection limits see Materials and Methods).

Table 2. END concentration in suspended particles: Peak samples were related to the 184-mm rainfall event on March 1, 2001; post-peak samples were taken 2 weeks after the rainfall event

Samples	Stream	Type of sample	END (μg/kg)
Peak	Maguire	Suspended particles	nd[a]
	Horqueta	Suspended particles	30
	Horqueta	Flood suspended particles	318
	Helves	Runoff sediments	10
	Helves	Suspended particles	43
Postpeak	Maguire	Suspended particles	nd
	Horqueta	Suspended particles	nd
	Helves	Suspended particles	nd

[a] Not detected (<2 μg/kg).

Macroinvertebrate Species Density

The macroinvertebrate communities were sampled on four occasions, at 3-week intervals. The mean number of species for each sampling is shown in Figure 1. The total number of species found throughout this study was 33 in Maguire, 26 in Helves, and 40 in Horqueta. The communities were mainly composed of species belonging to the orders Decapoda, Amphipoda, Ephemeroptera, Odonata, Diptera, Trichoptera, and Gastropoda.

Between the second and the third sampling date, a significant (ANOVA; $p = 0.01$ and $p = 0.0009$) decrease in species density was detected in Horqueta and Helves. The total decrease amounted to 7 species in Horqueta and 10 in Helves. The vanishing species mainly belonged to the orders Ephemeroptera, Odonata, Decapoda, and Diptera. Maguire showed no decrease in species density.

Abundance of Ephemeroptera and Odonata

Ephemeroptera and Odonata comprised 19, 35, and 33% of the individuals at Maguire, Horqueta, and Helves, respectively. Of the three ephemeropteran species found in the three investigated streams, one belonged to the genus *Baetis* and two to the genus *Caenis.* The species *Hetaerina rosea, Oxyagion terminale,* and *Ischnura fluviatilis* were the most abundant odonatan species in the three streams.

In Maguire, the Ephemeroptera did not vary significantly over the whole sampling period (Fig. 2). A significant decrease in Odonata occurred from the first (05.02.01) to the second (26.02.01) sampling, followed by a significant increase in the abundances by the third (19.03.01) sampling date. Horqueta and Helves showed a different pattern in odonatan abundances. There was a slight increase in abundances of Ephemeroptera in Horqueta and Helves and of Odonata in Helves from the first (05.02.01) to the second (26.02.01) sampling, whereas the Odonata in Horqueta slightly decreased. Most prominent was a significant decrease in the abundances of Ephemeroptera from the second (26.02.01) to the third (19.03.01) sampling date in Horqueta (from 338 ± 110 to 44 ± 7 individuals/m^2; ANOVA, $p < 0.0001$). In Helves they disappeared in the samples of the third sampling date (from 100 ± 84 to 0 ± 0 individuals/m^2; $p = 0.008$). The order Odonata (Fig. 2) showed the same pattern, with a significant decrease in Horqueta (from 46 ± 20 to 6 ± 6 individuals/m^2; $p = 0.006$) and Helves (from 60 ± 26 to 0 ± 0 individuals/m^2; $p = 0.001$). In view of the fact that Ephemeroptera and Odonata at Helves were reduced to zero on the third sampling date, the subsequent increase in abun-

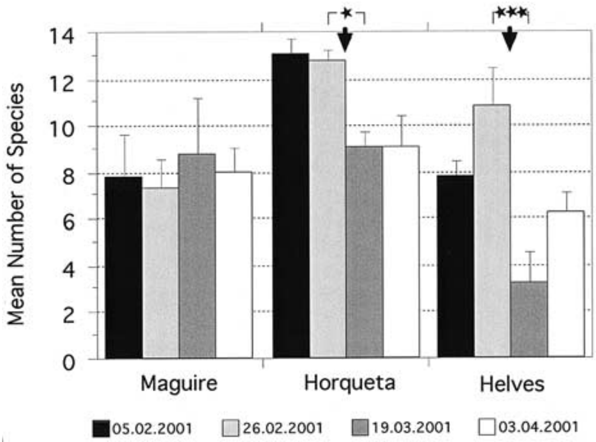

Fig. 1. Mean number (+SE; $n = 4$) of species in the three streams on each of four sampling dates. The arrows indicate a contamination with END in suspended particles of Horqueta and Helves. Asterisks indicate a significant difference between sampling dates (ANOVA; $^{*}p < 0.05$, $^{***}p < 0.001$)

dances of both groups at the fourth sampling date was again significant.

Macroinvertebrate Drift

The main groups of macroinvertebrates found in the driftnets were ephemeropteran nymphs belonging to the families Caenidae and Baetidae, individuals of the family Smicridae (Trichoptera), and *Hyalella curvispina* (Amphipoda).

The macroinvertebrate drift in Maguire was relatively low, varying between 2 and 22 individuals per 9 days throughout the sampling period (Fig. 3). In contrast, drift in Horqueta showed two distinct peaks: on 20.02.01, with 79 individuals, and on 07.03.01, with 50 individuals per driftnet (Fig. 3). Both peaks were associated with rainfall events of more than 100 mm/day. Ephemeroptera formed 27 and 60% of the drift during these two peaks, while the Trichoptera family Smicridae contributed 70% of the drift during the first peak but was not present during the second drift peak. In March, the Smicridae were also not detectable in the macroinvertebrate samples. During the second drift peak in Horqueta, *Hyalella curvispina* constituted 40% of the individuals (Fig. 3). No drift samples were obtained from Helves during the rainfall event at the beginning of March, because the high water level prevented the recovery of the driftnets for about 3 weeks (no figure).

Discussion

Runoff-Related Pesticide Contamination

Peaks of END contamination were clearly related to rainfall events that raised the water levels in the streams, which suggests edge-of-field runoff as a route of entry. Although cypermethrin was applied at higher rates, only END was detected in suspended-particle samples. This is possibly due to the high persistence of END within the aquatic environment (Goebel *et al.* 1982). However, it is likely that other pesticides, which were not analyzed in this initial study, were present in those samples that contained END. Moreover, high levels of particle-associated pesticide may result in increased concentrations of aqueous-phase pesticide, which may cause toxicity, as shown for END by Leonard *et al.* (2000). In this study Leonard *et al.* pointed out that rainfall in the locality of exposed sites and END concentration of the Namoi River was evidentially correlated.

The highest concentration of END, 318 μg/kg, was found during the present study in suspended particles from the flood sampler in Horqueta. Schulz (2001) reported total END (α, β, S) concentrations associated with suspended particles during runoff in the range of 179 to 12,082 μg/kg in the Lourens River of South Africa, which is surrounded by orchards. Following spray application, Wan *et al.* (1995) measured END concentrations in farm ditch sediments in Canada ranging from 5 to 2461 μg/kg. Leonard *et al.* (2000) measured up to 48 μg/kg of total END in sediments of the Namoi River in Australia.

The high END concentration in the event-triggered flood sampler in comparison with the continuously operating suspended particle sampler indicates the importance of short-term contamination during runoff events. Futhermore, END was present only in the samples taken during the rainfall period and was absent in the postpeak samples taken 2 weeks after the runoff event. Such a transient character of runoff-related contamination peaks had previously been reported in several other studies (Kreuger 1995; Williams *et al.* 1995; Schreiber *et al.* 1996).

No END was detectable in the suspended matter in Maguire during the runoff event in March. At this stream, the sampling site was located 300 m downstream of the nearest edge-of-field runoff input site, as indicated by erosion rills, and the water had to pass through a 0.055-ha wetland situated in between. In sediment that was collected from the bottom of the wetland, a concentration of END of 109 μg/kg was detected. It is hypothesized that the wetland through which the Maguire stream flows upstream of the sampling site is effectively reducing the END content due to processes such as sorption and sedimentation (Rodgers *et al.* 1999). Recently other studies have documented the retention of particle-associated insecticides downstream of a 0.4-ha vegetated wetland in South Africa (Schulz and Peall 2001; Moore *et al.* 2002).

Response of Macroinvertebrates

We observed a significant decrease in species density and a significant decrease in Ephemeroptera and Odonata abundances in Helves and Horqueta following a heavy rainfall associated with END contamination. In contrast, END was not detected in Maguire and this site showed no decline in species density, increasing Ephemeroptera abundances and maximum Odonata abundances coincided here with the population declines observed at Helves and Horqueta. It is likely that these differences in invertebrate dynamics are linked to the differences in pesticide contamination, as indicated by the END records.

END is highly toxic to fish and moderately toxic to aquatic

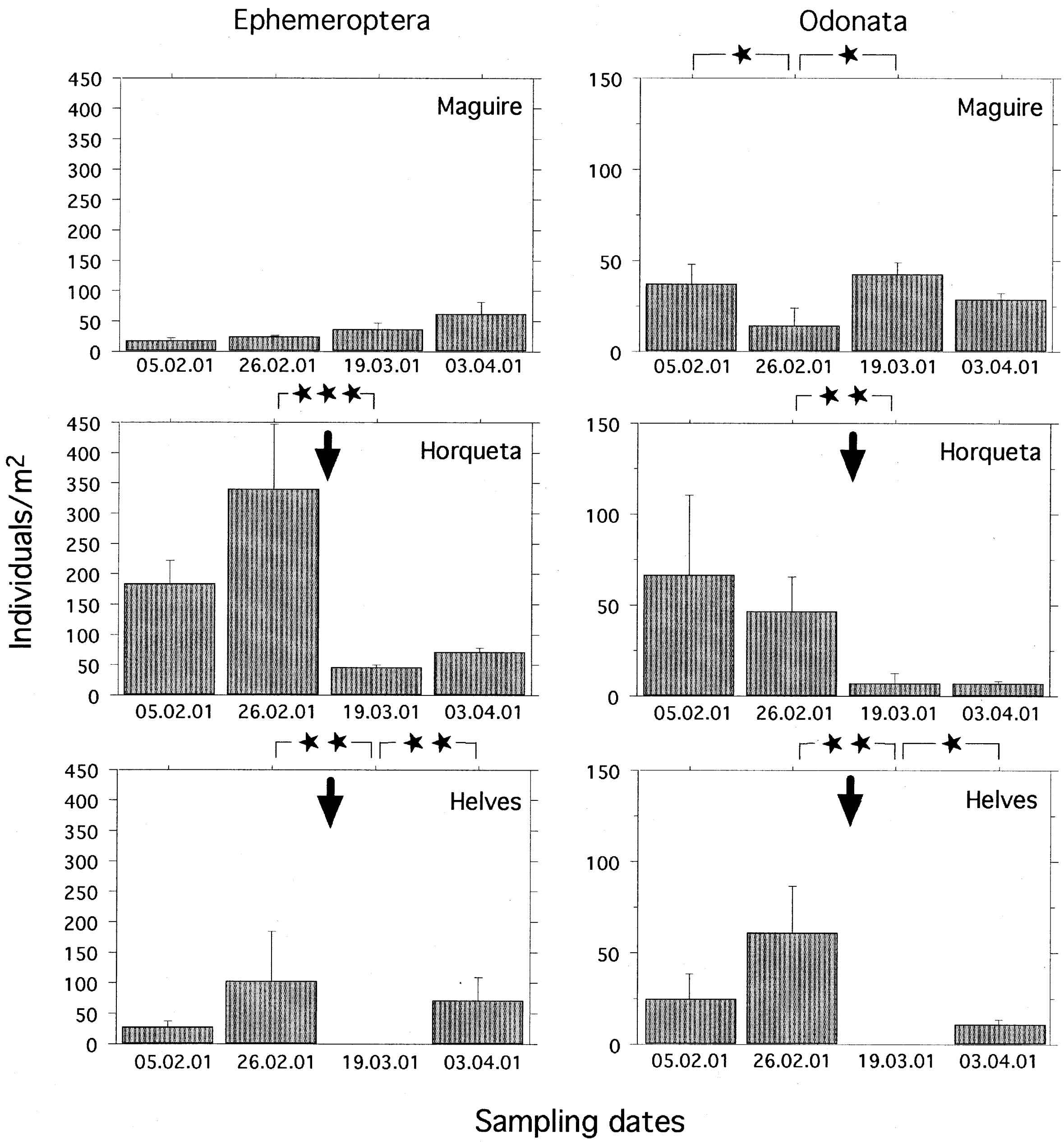

Fig. 2. Mean abundances (±SE; $n = 4$) of the order Ephemeroptera and Odonata. The arrows indicate a contamination with END in suspended particles of Horqueta and Helves. Asterisks indicate a significant difference between sampling dates (ANOVA; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$)

invertebrates. Ernst *et al.* (1991) found a 24-h LC_{50} of 8 μg/L for the three-spined stickleback (*Gasterosteus aculeatus*), 53 μg/L for *Chironomus plumosus,* 13 μg/L for the water boatman (*Sigara alternata*), and 72 μg/L for a caddisfly larva (*Limnephilus ssp*). A decrease in population densities of mayfly nymphs and caddisfly larvae was significantly correlated with aqueous-phase END, which varied from 13 to 911 μg/L (Leonard *et al.* 2000). In another study, Leonard *et al.* (2001) found a 10-day LC_{50} value for total END in a sediment toxicity test with *Jappa kutera* (Ephemeroptera) of 162 μg/kg. The concentration detected in the suspended particles of Horqueta during the present study was even higher. Leonard *et al.* (2000) inferred from their results that the densities of four species of Ephemeroptera and two species of Trichoptera were negatively correlated with the total END concentrations caused by runoff. It has been well established that Ephemeroptera and Odonata, the orders mainly affected in the present study, are highly sensitive to pesticide pollution (Mian and Mulla 1992; Siegfried 1993).

The fact that particle-associated pesticides may affect aquatic invertebrates has also been shown using stream microcosms. Schulz and Liess (2001b) found increased drift of

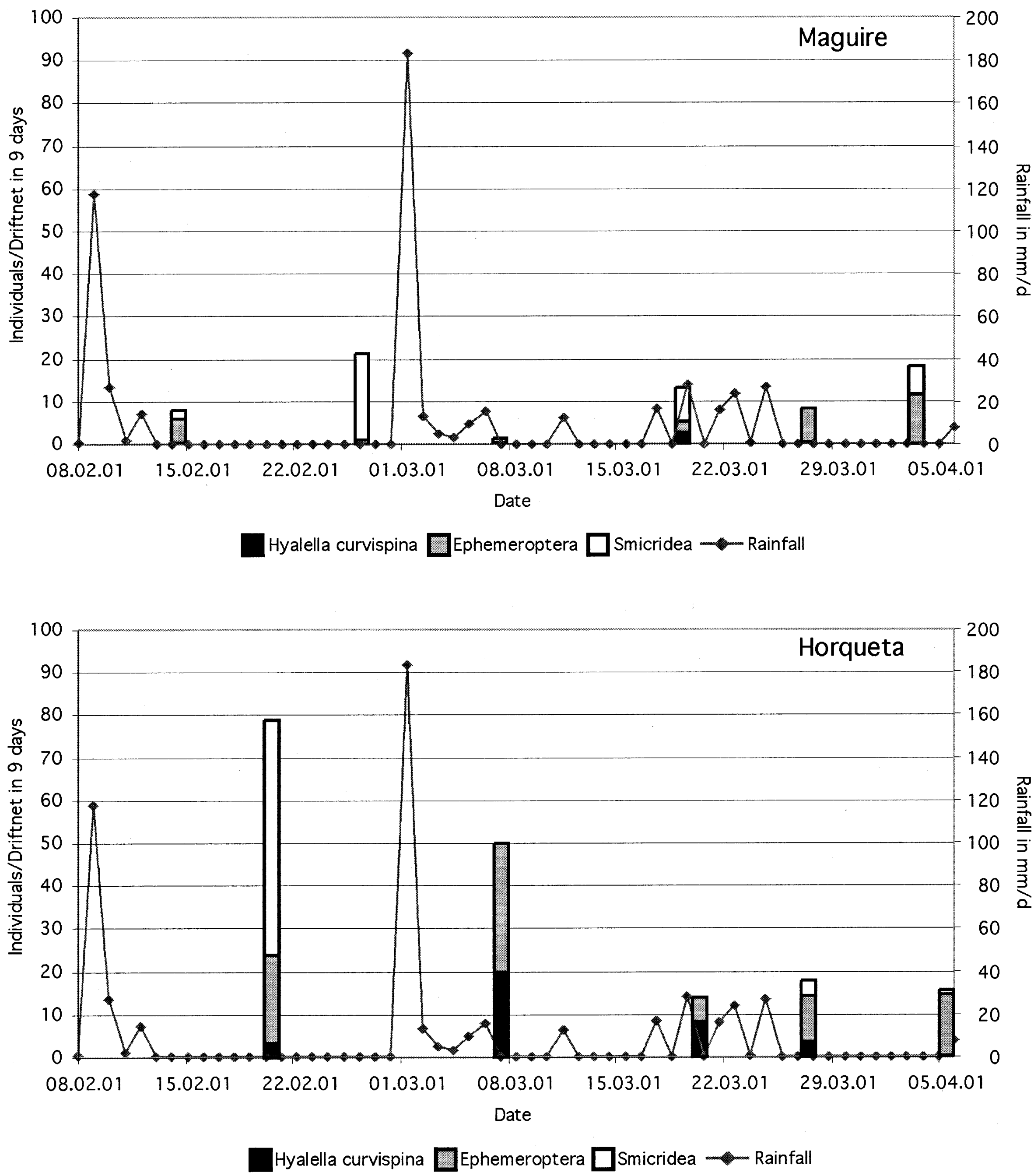

Fig. 3. Drift of macroinvertebrates in Maguire and Horqueta as well as rainfall data. Each bar represents the average drift in the time interval lasting from the next bar to the left until the date indicated by the position of the bar on the *x*-axis, i.e., about 20 individuals per 9 days drifted in the period between February 16 and 27 in Maguire

various invertebrate species at a concentration of 13.6 μg/kg and reduced survival of a caddisfly species at 136 μg/kg of the pyrethroid fenvalerate in the suspended particles added in a runoff simulation study. Although it has been reported that sorption of pesticides decreases their bioavailability (Coats *et al.* 1989; Chandler *et al.* 1994), particle-associated insecticides produce effects even at field-relevant levels (Schulz and Liess 2001a).

Drifting Macroinvertebrates

The drift peak of *Hyalella curvispina* and Ephemeroptera in Horqueta (Fig. 3) during the period of END contamination at the beginning of March is likely to be related to the pesticide pollution. The drift of macroinvertebrates in periods without pesticide contamination corresponded in Horqueta and Maguire within an average of 18 individuals per driftnet even during runoff events, while the drift peak in March showed a threefold higher drift of macroinvertebrates than the average in Horqueta. Organismic drift has been reported in various studies as a reaction to transient insecticide pollution (Cuffney *et al.* 1984; Kreutzweiser and Sibley 1991). Furthermore, it has been shown that amphipods and ephemeroptera nymphs are particularly present in the organismic drift during insecticide exposure (Breneman and Pontasch 1994; Schulz and Liess 1999). In Maguire, where no END contamination was detectable, the drift was close to zero during the strong rainfall event in March.

Another earlier drift peak was detected in Horqueta in February during the period with the first strong rainfall event, during which no samples were taken for pesticide analysis. However, this drift peak comprised mainly caddisflies belonging to the Smicridae. It is not possible to decide unequivocally whether this drift peak is also related to an insecticide-input event. However, it is not likely, as there was also a drift peak of Smicridae in Maguire between February 16 and 27, a period without any rainfall. Furthermore, it has been reported that caddisfly larvae do not necessarily show an increased drift reaction in the field during pesticide exposure (Schulz and Liess 1999). As the Smicridae showed a high drift rate in both streams regardless of any potential contamination, it is assumed that these drift peaks represent a distributional drift (Brittain and Eikeland 1988) rather than a reaction to toxic conditions.

When the drift data are combined with the macroinvertebrate species density and abundance data, it becomes obvious that the second drift peak in March, which is present in Horqueta but not in Maguire, is related to pesticide contamination. As already discussed above, the species density and the Ephemeroptera and Odonata abundances decreased significantly during the same period in Horqueta as well as in Helves, but not in Maguire. The first drift peak in Horqueta in February was not associated with an effect on the species density or abundance dynamics of this stream and the populations in Maguire also showed no decline despite an increased drift of Smicridae during February. However, the abundance of Smicridae decreased in Horqueta from 20 individuals/m^2 in the sampling before to 2 individuals/m^2 after the rainfall event on February 9. In March this family was almost absent from the macroinvertebrate community in Horqueta and it was also not found in the driftnet during the runoff event in early March.

On the other hand, it is possible that low pesticide concentrations during the February rainfall event caused only a sublethal drift reaction (Taylor *et al.* 1994) and did not produce a population effect, whereas severe pesticide pollution in March produced a drift reaction that was associated with a population decline. Sibley *et al.* (1991) described invertebrate drift caused by permethrin contamination as low as 0.35 μg/L, while population effects were present only at sites with concentrations above 1.7 μg/L.

Conclusion

Non-point-source END pollution following storm runoff affected the macroinvertebrate community in agricultural streams in the Argentine pampa. Other potential routes of pesticide entry into surface waters, such as spray drift, should also be investigated, to provide data for a thorough risk assesment of pesticides in this region. Furthermore, the potential pesticide effects on fish are unknown in this area, although fish kills have been observed in the past. Strategies suitable under the local conditions for improving water quality and protecting the water resources should be developed and implemented. Vegetated wetlands as in Maguire seem to be one potentially effective means of mitigating the risk associated with agricultural pesticide contamination.

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