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Rapid Communication

Linking in situ bioassays and population dynamics of macroinvertebrates to assess agricultural contamination in streams of the Argentine pampa

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Abstract

The two local crustacean species *Hyalella curvispina* and *Macrobrachium borelli* were chosen for assessment of agricultural contamination in two streams (Horqueta and Maguire) in the Argentine pampa. In parallel with in situ bioassays of both species, the population dynamics and the organismic drift of *H. curvispina* were investigated throughout the main period of insecticide application, from December 2001 to March 2002. In Maguire none of the current-use insecticides (chlorpyrifos, α-cypermethrin, and endosulfan) in question were detected throughout the sampling period. During 1-week intervals with no contamination by insecticides the survival rate of *H. curvispina* varied between $77\pm6\%$ ($\pm$SE, $n=4$) and $85\pm3\%$. In Horqueta during a week with a peak insecticide contamination of 64 μg/kg chlorpyrifos in the suspended particles, a mortality of 100% was observed in the in situ bioassays for both species, *H. curvispina* and *M. borelli*. At the same time, in Maguire *H. curvispina* showed reduced survival rates of $23\pm5\%$ and $25\pm18\%$ at the two sites, while the survival rate of *M. borelli* was $60\pm11\%$ upstream and $93\pm5\%$ downstream, below a wetland. During the period with 100% mortality of *H. curvispina* in Horqueta, the population density of this species decreased correspondingly, from 106 ± 26 to 0 individuals/m^2. We conclude that in situ bioassays can be successfully linked to in-stream population dynamics for the same species and that this link is very useful for interpreting causal exposure–effect relationships.

Keywords: In situ bioassay; *Hyalella curvispina*; Chlorpyrifos; Population dynamics; Drift

1. Introduction

The ecological assessment of environmental contamination requires the use of toxicological tests at different levels of biological complexity (Cairns Jr. et al., 1994). Specific approaches that meet these requirements have been developed, standardized, and validated throughout recent decades. Since most of this work was done in the United States and Europe, the methods thus developed may need to be adapted to different local situations and species, e.g., in the Rio de La Plata catchment in Argentina. In this study an in situ bioassay was used to assess the pesticide contamination of agrarian streams in the area of intensive soybean farming within the Argentine pampa. Various local species were chosen (*Hyalella curvispina* (Amphipoda), *Macrobrachium borelli* (Decapoda), *Heterina rosea* (Odonata), and *Aegla uruguayana* (Decapoda)) to determine a test organism which is easy to keep in the laboratory and/or to handle in the field experiments. The species *H. curvispina* as a representative of the local communities turned out to be a test organism suitable for in situ bioassays because of its easy maintenance and high abundance in some local streams. In laboratory bioassay tests *H. curvispina* were used by Di Marzio et al. (1999) to assess the toxicity of

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wastewater sludge. Graça et al. (2002) performed laboratory tests and in situ bioassays with this species to assess urban pollution near the city of La Plata, Argentina. *H. azteca* is used as a standard test organism to assess the contamination of water and sediment (Chappie and Burton Jr., 1997; Hatch and Burton Jr., 1999; US EPA, 2000). However, biotests with *H. curvispina* have not yet been successfully linked to agricultural pesticide pollution. To extrapolate from bioassays to the field it is important to assess the ecological validity of the test results. Schulz and Liess (1999b) were able to show that bioassays using *Gammarus pulex* (Amphipoda) and *Limnephilus lunatus* (Trichoptera) may overestimate toxicity in the field, since the caged amphipods were prevented from using their usual downstream drift to avoid contamination. However, in this study the survival rates of *G. pulex* and *L. lunatus* were significantly reduced in the in situ bioassays after insecticide contamination.

Since the introduction of transgenic soybean in Argentina in 1997, production of this crop is growing rapidly with increasing harvests every year. In 2001 the soybean production reached 23.96 million metric tons on a harvested area that had expanded to 10.26 million hectares (INDEC, 2001). This expansion was accompanied by increased pesticide application, from 39.3 to 124 million kg between 1991 and 1997 (Pengue, 2000). Hence nontarget aquatic systems also risk greater exposure, which needs to be assessed using suitable field methods. The streams used in this study run through the main region of soybean production in the province of Buenos Aires. Of the total amount of insecticides used in the soybean area of Buenos Aires, the pyrethroid cypermethrin accounts for 50%, followed by the organophosphate chlorpyrifos, the pyrethroid deltamethrin, and the organochlorine endosulfan (oral communication from, Instituto Nacional de Tecnología Agropecuaria, Pergamino).

The aim of this study was to assess insecticide pollution in Argentine streams using in situ invertebrate bioassays and to link the bioassay results with the population dynamics of the same species in the streams to facilitate the interpretation of exposure–effect relationships.

2. Materials and methods

2.1. Study sites

The two studied streams, Maguire and Horqueta, are located near the city of Arrecifes in the province of Buenos Aires and flow into the Rio Arrecifes, which belongs to the Rio de La Plata catchment. Each stream was sampled at its intersection with the Route No. 8, 150–196 km from Buenos Aires. They run through an area, in which 80% is in agricultural use. About 80% of the planted crop consists of soybeans. Horqueta is a second-order stream, characterized as follows: 2–6 m width, 0.3–0.7 m depth, 0.1–0.4 m^3/s discharge, drainage basin of 500 ha. It has developed a floodplain containing many emerged and submersed macrophytes. Maguire ranges between 2 and 4 m in width and between, 0.3 and 0.8 m in depth with a discharge of 0.1–0.3 m^3/s and a drainage basin of 380 ha. It flows through a wide, shallow area covered with dense stands of emerged macrophytes, mainly bulrush (*Schoenoplectus californicus*), forming a small wetland of 30×18 m (ca. 0.055 ha). Two sampling sites were chosen in Maguire for the in situ bioassays and the macroinvertebrate sampling. One site in Maguire was located 300 m downstream of the wetland. It was expected that here, below the wetland and a following wide region with a very low water velocity, the contamination would be reduced due to sorption and degradation processes (Schulz and Peall, 2001). An exposed site of Maguire that was located 200 m upstream of the wetland received runoff water from the fields. At the site upstream the wetland of Maguire and at the site in Horqueta, edge-of-field runoff potentially enters the water bodies from the adjacent agricultural fields, as indicated by erosion rills. The sampling took place during the main period of insecticide application to the soybean crops, from November 2001 to February 2002.

2.2. Test organisms

H. curvispina and stream water were collected from Horqueta 500 m upstream of the sampling site where the stream runs for 1 km through an area of pasture and grassland. Individuals of *H. curvispina* were kept in the lab in 2-L beakers filled with stream water for at least 2 weeks before in situ testing. During this period, a piece of plastic mesh served as a substrate; the beakers were aerated constantly and oxygen, pH, and conductivity were measured twice a week. Once a week the water was renewed. *H. curvispina* was fed with a mixture of yeast, trout chow, and alfalfa leaves every second day as recommended by the guidelines of US EPA (2000) Additional Tetrafin and microcell (dried algae) were added once a week to the food. As a validity criterion, a mortality of less than 20% is considered no effect in the in situ bioassays, as in the 10-day sediment tests with *H. azteca* (US EPA, 2000). Chappie and Burton Jr. (1997) reported a survival rate greater than 80% for in situ bioassays with *H. azteca* after 4 weeks of stream exposure. Adult individuals 10–15 mm in body length were chosen for the in situ bioassays. *M. borelli* was collected at the same site as *H. curvispina* in Horqueta and was transferred immediately to the test chambers which were then located at the sample sites. The preliminary period in the lab was eliminated

for *M. borelli* because this species turned out to be sensitive to changes in temperature and oxygen during transport, causing some mortality. Juvenile individuals with a body length of 3–4 cm were used for the in situ bioassays.

2.3. Test chambers and handling

The chambers were constructed from white plastic tubes 5 cm in diameter and 15 cm long. The ends were closed with fitted plastic caps. Two rectangular windows measuring 3×5 cm on opposite sides of the chambers, which were covered with plastic 1.5-mm mesh, allowed exposure to stream water and suspended particles. To prepare the in situ bioassays the chambers were placed in containers with stream water and 10 *H. curvispina* were added below the water surface with a glass pipette. A piece of *Elodea* sp. was added as a source of food and as a substrate. Four replicates were placed together at each sampling site on the streambed with one window facing the bottom to allow contact with the sediment. The opposite window was exposed to the water column (Chappie and Burton Jr. 1997). The chambers were covered with dark, pervious plastic tissue to reduce exposure to sunlight. After the exposure period of between 6 and 8 days the chambers were opened and the surviving organisms counted.

2.4. Pesticide sampling

The suspended particles transported in the water phase of the stream were accumulated continuously by suspended-particle samplers (Liess et al., 1996), which consisted of 3-L glass bottles firmly attached to sticks and installed at each sampling site at the bottom of the streams with inlet (opening: 10×3 mm) and outlet pipes. The retention efficacy for the particles suspended in the stream water in the grain-size fraction below 0.02 mm averaged 50% at 0.05 m/s and 15% at 0.41 m/s flow velocity (Liess et al., 1996). The samplers were emptied weekly from the end of November 2001 through February 2002.

Water and soil particles washed into the stream from the fields during rainfall were sampled with specific runoff samplers (Schulz et al., 1998). They consisted of 3-L glass bottles buried in the soil up to their necks in erosion rills between the agricultural fields and the surface water of the streams.

2.5. 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. Extracts were eluted from 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, following methods described in Schulz et al. (2001). Detection limits in sediment and water were as follows: for chlorpyrifos and α- and β-endosulfan, 2 μg/kg and 0.01 μg/L; for α-cypermethrin, 5 μg/kg and 0.05 μg/L. 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 by standard methods (APHA, 1985).

2.6. Population dynamics and drift of H. curvispina

Four replicate samples from vegetated areas were taken monthly (04.12.2001, 09.01.2002, 07.02.2002, and 05.03.2002) in each stream with a surber-sampler (sampled area 0.125 m^2, mesh size 1 mm). All *H. curvispina* captured within the sampler were counted, preserved, and identified. Drifted *H. curvispina* were sampled by means of cylindrical plastic nets (20 cm diameter opening, 1 m long, 1 mm mesh size) installed in each stream at the bottom in duplicate. All macroinvertebrates that were captured within a 7-day period were counted, preserved and identified. During the sampling period 10 samples were taken in Maguire and 10 in Horqueta.

2.7. Data analysis

Differences in the in situ survival of *H. curvispina* during periods of insecticide contamination and periods without contamination were analyzed using one-way analysis of variance followed by Fisher's protected least-significant-difference test.

The same test was used for comparison of survival rates of *M. borelli* between all sampling sites in the period 15.01.02–23.01.02 and for the sampling dates of the stream population of *H. curvispina*.

3. Results

3.1. Insecticide contamination

The input of insecticides to the Horqueta occurred during three runoff events in January and at the beginning of February. The rainfall of 93.5 mm/day on the 5th of January was associated with introductions of 150 μg/kg chlorpyrifos, 46 μg/kg α-cypermethrin, and 7.8 μg/kg α- and β-endosulfan in the runoff sediment (Table 1). In the runoff water and the suspended particles of the stream only chlorpyrifos was detected, with concentrations of 0.3 μg/L and 7.7 μg/kg, respectively. Samples of runoff sediment resulting from the

Table 1
Insecticide concentration in Horqueta

Type of sample	Sampling date	Chlorpyrifos	α-Cypermethrin	Endosulfan (α and β)
Runoff sampling				
Runoff sediment (μg/kg)	08.01.2002	150	46	7.8
	29.01.2002	43	53	ND
	07.02.2002	15	13	ND
Runoff water (μg/L)	08.01.2002	0.3	ND	ND
	29.01.2002	0.09	ND	ND
	07.02.2002	0.07	ND	ND
In-stream sampling				
Suspended particles (μg/kg)	08.01.2002	7.7	ND	ND
	23.01.2002	64	ND	ND
	29.01.2002	11	ND	ND
	07.02.2002	ND	ND	ND

ND, not detected.
On three of the sampling dates (08.01.02, 29.01.02, and 07.02.02) there were rainfall (94, 34, and 85 mm/day) and runoff events. In the period from 14 to 23.01.02 there was no rain.

rain on the 26th of January contained 40 and 43 μg/kg chlorpyrifos and 25 and 53 μg/kg cypermethrin. In the suspended particles a concentration of 11 μg/kg chlorpyrifos was found, with 0.09 μg/L chlorpyrifos in the runoff water. In the runoff samples taken during the rain event on 31.01.02 (85.3 mm/day), a concentration of 15 μg/kg chlorpyrifos and 13 μg/kg cypermethrin in the sediment and 0.07 μg/L chlorpyrifos in the water was detected (Table 1). Cypermethrin was present only in the sediment of the runoff, being undetectable in the water phase or suspended particles, while chlorpyrifos was detected in the runoff sediment and in smaller amounts in the suspended particles. The highest concentration of chlorpyrifos (64 μg/kg) in samples from the stream itself was found in the suspended particles in Horqueta on the 23rd of January in a period with no rain. No insecticides were detected in the suspended particles or water samples at either of the sampling sites in Maguire.

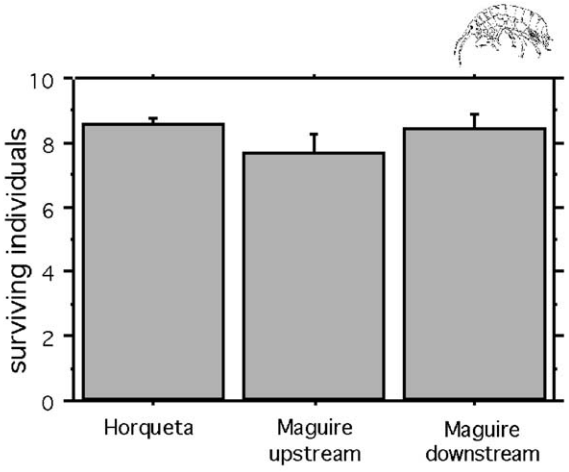

Fig. 1. Mean number of surviving individuals (±SE; $n = 12$) of *H. curvispina* at the three sampling sites in weeks with no contamination.

3.2. Nutrients

The average concentrations (±SE; $n = 11$) in Horqueta were 0.23±0.04 mg/L for phosphate, 0.06±0.01 mg/L for ammonium, and 1.2±0.1 mg/L for nitrate. In Maguire the corresponding concentrations were 0.13±0.03 mg/L for phosphate, 0.02±0.01 mg/L for ammonium, and 1.7±0.2 mg/L for nitrate. All concentrations were well below toxic limits.

3.3. In situ bioassays: control intervals

Four in situ bioassays with *H. curvispina* were performed simultaneously in Maguire and Horqueta throughout the sampling period in January and February. In weeks with no runoff and insecticide concentrations below the detection limits the survival rate of *H. curvispina* was 77±6% (±SE) in Maguire upstream of the wetland, 84±4% in Maguire downstream of the wetland, and 85±3% in Horqueta (Fig. 1).

3.4. In situ bioassays: exposure intervals

Between 15 and 23 January, a significant 100% mortality occurred in the in situ bioassays with *H. curvispina* (Fig. 2b) and *M. borelli* (Fig. 2a) in Horqueta. During the same time interval a chlorpyrifos concentration of 64 μg/kg in the suspended particles was measured in Horqueta. No runoff event occurred during this time period, so that pesticide spraying by airplane is likely to be the cause of the measured contamination. The survival rate of *H. curvispina* was reduced significantly in Maguire at both sites with 23±5% and 25±18%. *M. borelli* showed a reduced survival rate of 60±11% at the upstream site of Maguire and a high survival rate of

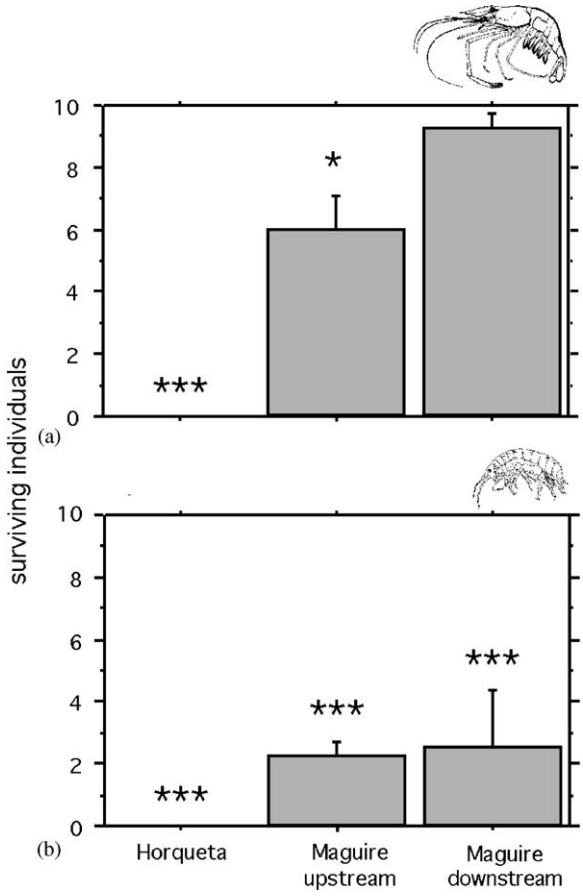

Fig. 2. Mean number of surviving individuals (±SE, $n=4$) of *M. borelli* (a) and *H. curvispina* (b) during a period (15.–23.01.02) with high chlorpyrifos contamination of 64 μg/kg detected in Horqueta. The significance levels for (a) refer to the comparison of survival rates at the Maguire downstream site. The significance levels for (b) were calculated in comparison with survival rates during a period with no contamination as shown in Fig. 1 (ANOVA Fischer's PLSD; $^{*}P<0.05$; $^{***}P<0.001$).

93±5% at the downstream site, during the period 15–23 January.

3.5. Linking bioassay results and population dynamics of *H. curvispina*

The abundance of *H. curvispina* in Horqueta varied slightly, between 106±26 ($n=4$, ±SE) and 103±55 individuals/m^2 between 04.12.01 and 09.01.02 (Fig. 3b). On the sampling occasion 1 month later (07.02.02) the density of *H. curvispina* decreased significantly to zero; is species also did not appear in the last sampling on 05.03.02. No significant changes in the population density of *H. curvispina* were observed in Maguire (not shown), although the densities were generally much lower, with an average of 3±1 individuals/m^2. The decrease in population density between 9 January and 7 February coincided with the 100% mortality in the in situ bioassays between 15 and 23 January. However, the in situ survival rates of this species during the later test intervals in February were again high, at 93±4% and 87±2% (Fig. 3a).

3.6. Drift of *H. curvispina*

Between 05.12.01 and 02.01.02 the drift of *H. curvispina* was relatively low, with between 2 and 22 individuals per driftnet on average. An increased drift with an average of 99 individuals per driftnet was observed between 1 and 8 January during a period with runoff on 5 January (rainfall of 94 mm/day; Fig. 4). The runoff was associated with a concentration of 7.7 μg/kg chlorpyrifos in the suspended particles and 150 μg/kg chlorpyrifos and 46 μg/kg α-cypermethrin in the runoff sediment (Table 1). Even 1 week later the drift was still elevated, with 44 individuals per driftnet (Fig. 4) and a high percentage (83%) of young individuals. The following runoff events did not cause a higher drift.

4. Discussion

4.1. Insecticide results

The insecticides endosulfan, α-cypermethrin, and chlorpyrifos were detected in the runoff sediment in Horqueta, ranging between 15 and 150 μg/kg. Only chlorpyrifos was found in stream samples, at concentrations ranging from 7.7 to 64 μg/kg in the suspended particles. Schulz et al. (2001) observed chlorpyrifos in the Lourens River, South Africa in suspended sediment after the first seasonal runoff, at concentrations from 15.5 to 344 μg/kg. During the present study, however, the highest chlorpyrifos concentration in the suspended particles was detected in a period without runoff events. The most likely alternative source in this case is airplane fumigation, resulting in insecticide deposition on the water surface and subsequent sorption to suspended particles. It is a general management practice in this region to spray the soybean fields by air after the plants reach a certain size because airplanes cause less damage to the crop than vehicles. Spraying airplanes were regularly observed during this period in the region. The detection of chlorpyrifos in suspended particles in Horqueta suggests that both edge-of-field runoff and spray deposition are relevant routes of insecticide entry into the stream, as has previously been shown for endosulfan (Jergentz et al., 2004).

4.2. In situ bioassays

The average survival rate of *H. curvispina* in periods with no detectable insecticide contamination was over 80% in Horqueta and the downstream site in Maguire (85±3% and 84±4%, respectively). Using in situ

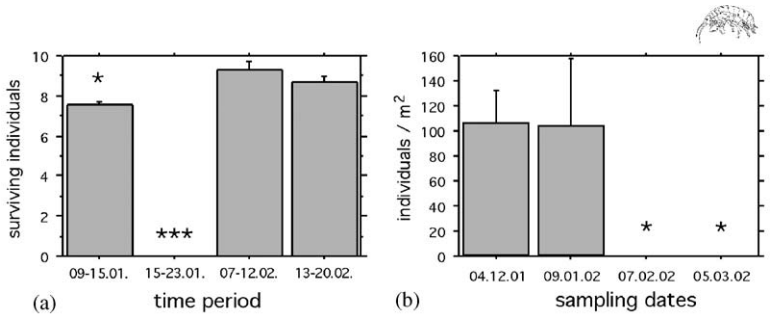

Fig. 3. Mean number of surviving individuals (±SE, $n = 4$) in in situ bioassays (a) and mean (±SE, $n = 4$) in-stream population densities (b) of *H. curvispina* in Horqueta. Significance levels were calculated between each time period or sampling date (ANOVA Fischer's PLSD; $^{*}P<0.05$; $^{***}P<0.001$).

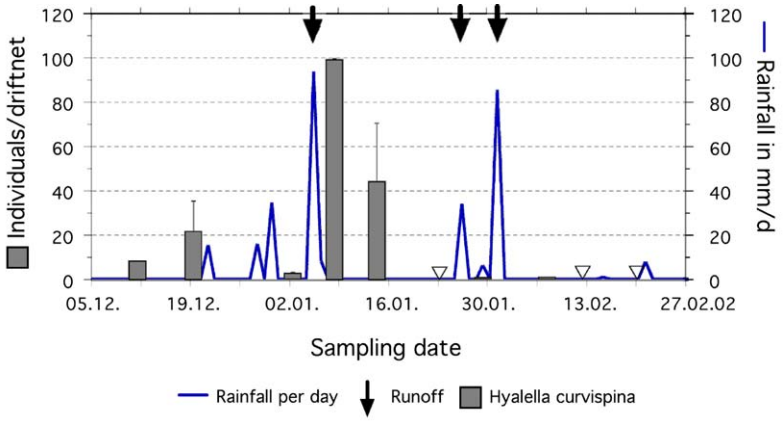

Fig. 4. Mean of drifting individuals (±SE, $n = 2$) of *H. curvispina* in Horqueta. The triangles indicate sampling points at which the drift of *H. curvispina* was zero.

bioassays with *H. azteca*, Chappie and Burton Jr. (1997) found survival rates greater than 80% in agricultural streams in the Little Miami River after an exposure up to 4 weeks. In in situ bioassays with the amphipod *Paramelita nigroculus*, Schulz (2003) observed survival rates greater than 90% in no-runoff periods (3–6 days) in two agricultural rivers in Western Cape in South Africa. The results presented in this study for the sites in Horqueta and Maguire (downstream) fulfill the validity criterion of the sediment standard test of the US EPA (2000) with *H. azteca* for a 10-day exposure in the laboratory. The in situ bioassay during 15–23.01.02, when contamination of the suspended particles in Horqueta amounted to 64 μg/kg chlorpyrifos, resulted in a significantly lower survival rate than those during all the other exposure times. Bioassays in Maguire showed a significantly lower survival rate of *H. curvispina* during the same time period, namely 23±5% at the upstream site and 25±18% at the downstream site, while the survival rate of *M. borelli* was reduced to 60±11% at the upstream site. It is likely that the high chlorpyrifos contamination of 64 μg/kg in Horqueta was toxic to the exposed organisms. Green and Chandler (1996) found sediment-associated toxicities (LC50) of chlorpyrifos to the marine copepod *Amphiascus tenuiremis* of 66, 74, and 40 μg/kg for adult, copepodite, and nauplius stages, respectively. With meiobenthic estuarine copepods the sediment-associated concentration of chlorpyrifos varied from 21 to 33 μg/kg for 25% lethal concentration of adult copepods and 50% lethal concentration of larval stages (Chandler et al., 1997). Graça et al. (2002) used in situ bioassay with *H. curvispina* to assess urban pollution in streams near La Plata, Argentina. In in situ bioassays with *H. azteca* Tucker and Burton (1999) found a decreased mortality response of this species in urban areas and increased mortality at agricultural sites when the results were compared with laboratory exposures. However, these studies with *Hyalella* species were not able to link effects in the in situ bioassays with the insecticide pollution of the streams. Moreover they had not the aim to compare effects in in situ bioassays with the dynamics of the stream population of the test organisms. On the other hand, several tests with amphipods and other crustaceans have pointed out the usefulness of these groups in bioassays during pesticide exposure (Baughman et al., 1989; Matthiesen et al., 1995; Schulz and Liess, 1999b; Schulz, 2003). Even in laboratory tests, crustaceans

appeared to be very sensitive to the detected insecticides. Van Wijngaarden et al. (1996) found a 96-h EC50 for *G. pulex* of 0.02 μg/L chlorpyrifos. In a comparative toxicity test with chlorpyrifos in the laboratory *H. azteca* was recognized as a sensitive invertebrate with a LC 50 (48 h) of 0.1 μg/L (Moore et al., 1998). Leight and van Dolah (1999) found, in acute tests with various durations (48, 72, and 96 h), ranges of chlorpyrifos LC50 s between 5.2 and 0.3 μg/L in static and between 6.5 and 0.2 μg/L in static-renewal tests with the estuarine amphipod *Gammarus palustris*. It is not possible to explain the increased mortality of *H. curvispina* at both Maguire sites during 15–23.01.02 by any of the measured insecticides. However, it is unlikely that insecticides cause mortality in the downstream site of Maguire while the survival rate observed in the bioassays with *M. borelli* was high.

It is assumed that sediment and nutrient load of the streams have little effect on the test organisms. Runoff events are associated with higher sediment and nutrient contents in the streams, but the highest mortality appeared in the in situ bioassays even in periods without runoff. The average concentrations of nutrients were below toxic limits.

4.3. Linking bioassay results and effects on the stream population of H. curvispina

During the month following the first sampling of the in-stream *H. curvispina* populations in Horqueta, none of the analyzed insecticides was detected in runoff or in suspended particles. Even in the second sampling (09.01.02), which was performed 4 days after the heavy rainfalls on the 5th of January with an introduction of insecticides, no changes in the population of *H. curvispina* were observed in comparison with the sample taken 1 month before. This implies that the runoff event on 5 January had no acute effect on the *H. curvispina* populations. However, the drift of *H. curvispina* on 8 January was likely to be related to the insecticide contamination during the rainfall on 5 January, when chlorpyrifos, cypermethrin, and endosulfan were present in the runoff sediment and chlorpyrifos was present in the suspended particles. The high drift of *H. curvispina* lasting until 14.01.02 may indicate a sublethal response caused by the runoff event. Organismic drift has been reported in various studies as a behavioral reaction to transient insecticide pollution (Cuffney et al., 1984; Kreutzweiser and Sibley, 1991). Furthermore, it has been shown that particularly amphipods are present in the organismic drift during insecticide exposure (Breneman and Pontasch, 1994; Schulz and Liess, 1999a) and they may use this behavior as an avoidance reaction. It is likely that the pesticide concentrations during this rainfall event were so low that only a sublethal drift reaction was triggered (Taylor et al., 1994) and no effect on population density was produced. Sibley et al. (1991) described invertebrate drift that resulted from permethrin contamination as low as 0.35 μg/L, whereas population effects were present only at sites with concentrations above 1.7 μg/L. There were three insecticide-input events between 09.01.02 and 07.02.02 that may be responsible for the complete breakdown of the population of *H. curvispina*. It seems most likely that the chlorpyrifos peak of 64 μg/kg in suspended particles caused the breakdown of the population of *H. curvispina* where spray drift is suggested as the way of entry. As mentioned above, the runoff on 5 January had no acute effect on the *H. curvispina* population, so it is unlikely that the following runoff events with insecticide concentrations at the same level caused such an acute reduction of the population.

Purely on the basis of the field results on drift and abundance, a direct link between insecticide contamination and effects remains rather speculative. However, the 100% mortality in the in situ bioassays coincided with the population breakdown in Horqueta and may thus help to identify causal exposure–effect relationships. On the other hand, the in situ bioassay on its own would not be sufficient to predict related effects on the population level in the field. In outdoor experimental ditches contaminated with chlorpyrifos the amphipod *G. pulex* appeared to be the most sensitive test organism (Brock et al., 1992a, b) with significant decrease resulting from the 0.9-μg/L treatment (Van den Brink et al., 1996; Van Wijngaarden et al., 1996). Werner et al. (2000) found in stream samples from the Sacramento–San Joaquin river delta toxicity of chlorpyrifos that caused 100% mortality in 48 h in bioassays with *Ceriodaphnia dubia* with a concentration of 0.125 μg/L. Examples for clear relationships between insecticide pollution and effects on macroinvertebrate dynamics in agrarian streams were given by Schulz and Liess (1999a, b) who found decreased abundances and increased drift of the aquatic fauna after a runoff-related insecticide input. In the present study it is likely that the mortality in the in situ bioassays, the population breakdown, and the drift reaction of *H. curvispina* were caused by insecticide pollution, namely the chlorpyrifos peak of 64 μg/kg. Jergentz et al. (2004) showed a decrease of mayfly and odonate species density in Horqueta following runoff-related endosulfan input. During this study, fewer individuals of *H. curvispina* were found in the driftnets during the 1-week intervals following the high drift observed during the first half of January. This is probably related to the high concentration of chlorpyrifos in the suspended particles on the 23rd of January and the 100% mortality of *H. curvispina* in the in situ bioassays. No individuals of *H. curvispina* were found in Horqueta after the population breakdown, whereas the survival rate in the in situ bioassays increased again to above 80% in later periods without insecticide

contamination, indicating the transient toxicity of the non-point-source insecticide pollution.

In summary, this study highlights the advantage of linking in situ bioassays with investigation of the effects of insecticide contamination on stream populations. The transient and toxicologically relevant presence of insecticide contamination in the field can be monitored using in situ bioassays while the population dynamics in the field give additional information on the long-term ecologically relevant effects on invertebrate populations.

5. Conclusions

In this study it is shown that in situ bioassays with the local species *H. curvispina* and *M. borelli* are a sensitive tool to assess insecticide contamination in agrarian streams in the Argentine pampa. In combination with information on population dynamics and drift reaction, the detection of relevant contamination events was enhanced and showed corresponding effects. In situ bioassays may be considered as part of the risk evaluation for pesticides in the streams that receive contaminants from fields under intensive agricultural use in Argentina.

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