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RESEARCH ARTICLE

The impact of pesticides on the macroinvertebrate community in the water channels of the Río Negro and Neuquén Valley, North Patagonia (Argentina)

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Abstract

Agriculture represents the second most important economic activity in the North Patagonian Region of Argentina and non-selective insecticides are still being used with significant implications to the quality of the environment. The range of concentrations (μg/L) determined for azinphosmethyl, chlorpyrifos, and carbaryl in drainage channels were from non-detected to 1.02, 1.45, and 11.21, respectively. Macroinvertebrate abundance and taxon richness in drainage channels were significantly lower in November compared to the other sampling months (October, February). The decrease in taxon richness observed in November was associated with chlorpyrifos and azinphosmethyl peak concentrations. The most remarkable changes were the decrease in sensitive taxa such as Baetidae and the increase in some tolerant taxa such as Chironomidae and Gastropoda.

For all three pesticides, the acute hazard quotient exceeded the risk criteria for invertebrates. The effects of the three pesticides on aquatic organisms, characterized by joint probability curves, showed that the LC_{50} of 10% of the species were exceeded five and three times by the concentrations of azinphosmethyl and chlorpyrifos during the study period, respectively. However, the correlation between the pesticide concentrations and both taxon richness and abundance of macroinvertebrates at each site (irrigation and drainage channels) was indicative that only chlorpyrifos was negatively correlated with both parameters (Spearman $r^2 - 0.61$, $p = 0.0051$ and Spearman $r^2 - 0.59$, $p = 0.0068$ for taxon richness and abundance correlation, respectively). We conclude that macroinvertebrate assemblages in drainage channels were highly affected by chlorpyrifos levels.

Keywords Agricultural land use · Aquatic pollution · Insecticides · Risk assessment · Macroinvertebrates · North Patagonia

Introduction

Agriculture represents the second most important economic activity in the North Patagonian Region of Argentina. Fruits hold the sixth position of the exported products by Argentina and, among them, the pomaceous represent 50% of those exports. About 95% of the exported apples and pears are produced in the Río Negro and Neuquén Valley (Sánchez et al. 2016). This area has a semiarid climate and requires a channel network derived from Limay, Neuquén, and Negro Rivers to manage water for agriculture. To complete the system, a drainage channel grid system returns the excess irrigation water to the nearby rivers. Both irrigation and drainage channels provide favorable habitats for many aquatic invertebrate species (Anguiano et al. 2008).

Despite integrated pest management implementation based on selective insecticides, biopesticides and sexual confusion

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technique; non-selective insecticides, such as carbamates and organophosphates are still used with significant implications to the environmental quality. In the Río Negro and Neuquén Valley, insecticides are applied every 15 days during the spring-summer period, with peak concentrations lasting a few hours before declining. Insecticides are applied to fruit trees by mist blowers, often contaminating both surface water and soil. In addition, artificial irrigation is performed by periodic flooding of the fruit orchards, which have a flat topography that would enable the transport of pesticides from the soil to surface and subsurface waters.

Studies conducted in the North Patagonia demonstrated the presence of organophosphates and carbamates in both ground (Loewy et al. 1999; Loewy et al. 2003; Loewy et al. 2006; Loewy et al. 2011) and surface (Loewy et al. 2011) waters. The organophosphates chlorpyrifos and azinphosmethyl showed similar detection frequencies of 73 and 76 %, respectively; meanwhile, carbaryl exhibited a detection frequency of 40% in the drainage channels (Loewy et al. 2011).

The transport of non-point source pesticides from agricultural areas is regarded as one of the main causes of water contamination which might occur by runoff, leaching, spray drift, preferential flow through soil macropores, or a combination of these processes (Phillips and Bode 2004; Gärdenäs et al. 2006; Loewy et al. 2011). Runoff is considered an important route of entry of pollutants to surface waters. Pesticide contamination of aquatic systems through runoff is dependent on a number of factors such as physicochemical properties of the pesticides, timing and rate of application, rainfall after pesticide application, and soil types (Phillips and Bode 2004).

Pesticides represent an important stressor for freshwater ecosystems and can impact all groups of organisms (Liess et al. 2008; Schäfer et al. 2012). Among aquatic organisms, macroinvertebrates organisms have been widely used to assess stream integrity since they possess several advantages compared to other groups of organisms (Infante et al. 2009; Miserendino et al. 2008). Macroinvertebrates can be considered excellent for biomonitoring aquatic systems since they are ubiquitous and diverse, exhibit different feeding habits, are sedentary, have life cycles ranging from few weeks to few years, show a tolerance range to contaminants, and possess a convenient size for field examination, storage, and transportation (Bonada et al. 2006). In addition, macroinvertebrate communities are sensitive to pesticides and are good indicators of overall ecosystem function (Overmyer et al. 2005; Wallace et al. 1996).

Since the drainage channels under study flow into the Neuquén River, the aim of this study was to evaluate the risk to aquatic organisms inhabiting these pesticide-contaminated waters by a tiered approach (ECOFRAM 1999), beginning with conservative assumptions and moving towards more realistic estimates of exposure and effects.

Methods

Site description

The Río Negro and Neuquén Valley, located in northern Patagonia (Argentina), is a privileged region for fruit production. The system under study involved a 110-ha section of an agricultural area near the Neuquén River, where 82% of this area is committed to apple and pear growing. This low-rainfall region is watered by a network of irrigation and drainage channels which discharge directly to the Neuquén River. The channel widths are approximately 1.5 and 0.7 m deep. Their flows are quite uniform, its variability depending on the irrigation regimes, without riffles or pools. Drainage water, mainly fed by filtered groundwater from aquifers, displays a higher volume during the irrigation period, with the Neuquén River the final receptor. It should be taken into consideration that the water from the downstream reaches of the Neuquén River is extracted for potable water purposes. The habitat of the channels was similar, with a dense riparian forest canopy composed of willows (*Salix* spp.) and poplars (*Populus* spp.) and the dominant substrates were pebbles, gravel, and sand, with little coverage of macrophytes, mainly *Stuckenia striata* and *Myriophyllum quitense*.

Sampling design

Pesticides were monitored over the pesticide application period (October to April) from 2008 to 2011 at five sites along the drainage channels and one site from an upstream irrigation channel (A, control site) in the studied area (Fig. 1). Samples collected at the site A were considered as a spatial control. The total number of samples was 108, including the control ones. In the latter two seasons, macroinvertebrate biomonitoring was performed in drainage and irrigation channels in October, November, and February. Three replicate samples were collected at each site with a Surber net sampler ($0.09\ m^2$, 250-mm mesh size) and fixed in situ with 4% formaldehyde. A total of 57 samples were sorted and analyzed at the laboratory under at least × 5 magnification. The macroinvertebrates were identified to the lowest possible taxonomic level using regional keys (Domínguez and Fernández 2009; Fernández and Domínguez 2001; Lopretto and Tell 1995). A set of macroinvertebrate community metrics such as taxon richness, total density, density of individual taxa (Gastropoda, Hyalellidae, Baetidae, Orthocladiinae, and Chironominae) and the Shannon-Weaver diversity index were determined.

Physicochemical conditions were measured before each sampling period with a multiparametric equipment (PASCO). Parameters measured in situ were water temperature (°C), pH, electric conductivity (μS/cm), and dissolved oxygen (mg/L). To measure the discharge of irrigation and

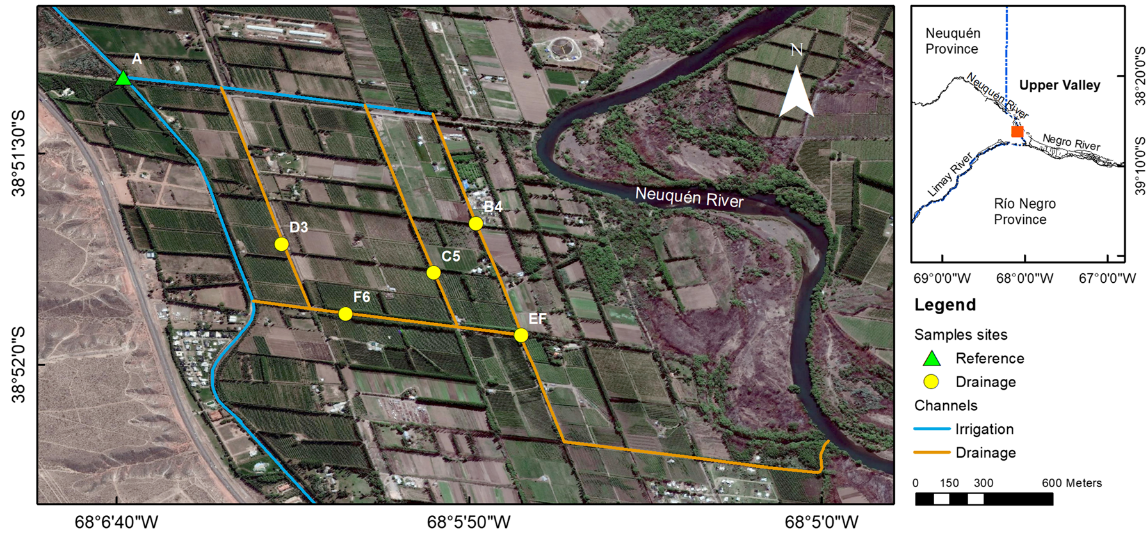

Fig. 1 Sampling sites in the Río Negro and Neuquén Valley (Argentina). Irrigation channel is labeled as A (reference), and drainage channels are labeled as B4, C5, D3, EF, and F6. Irrigation channels flow to the Neuquén River

drainage channels, the midsection method was used (Hauer and Lamberti 2011). Flow measurement equipment included a current meter (PASCO), a top-setting wading rod and a tape measure.

Analytical methods

Organophosphate and carbamate pesticides, known to be frequently used in the area under study, were investigated. Approximately 1 L of water samples were collected from the middle of the channels and from their mid-column. Samples were immediately refrigerated and transported to the laboratory where analysis was performed within 24 h of receipt. Samples were kept at 4 °C until analysis. Samples were filtered and analyzed by solid phase extraction using C18 columns (Strata Phenomenex), previously conditioned with 6 mL methanol followed by 6 mL of water. Subsequently, the pesticides were eluted with 2 mL hexane followed by 4 mL dichloromethane. The extracts were dried in a nitrogen stream and further dissolved in 0.25 mL hexane. Concentrated extract quantitation was performed with a gas chromatograph (Agilent 6890), splitless mode, provided with capillary column and nitrogen-phosphorus detector for organophosphates and carbamates (slightly modified from the method 3535A by USEPA 2015). The positive detections were confirmed with gas chromatography-mass spectrometry (GC-MS), provided with a PTV injector. The recovery percentages ranged between 70 and 110 %, with a variation coefficient lower than 12%. Linearity was measured by R^2 coefficient for the individual pesticide calibration curves, always resulting in ≥ 0.99. Each set of samples was analyzed in duplicate, simultaneously with a laboratory blank. The limit of detection (LOD) was below 0.07 and 0.20 μg/L for organophosphates and carbamates, respectively. On the other hand, the limit of quantitation (LOQ) was below 0.10 and 0.40 μg/L for organophosphates and carbamates, respectively.

Risk assessment

The risk of pesticides to aquatic organisms inhabiting drainage channels was assessed using a tiered approach according to the ECOFRAM (ECOFRAM 1999) Aquatic Report. A preliminary and deterministic assessment of the potential risk was calculated by the comparison of acute and chronic toxicity data from the literature to determine the worst-case scenario. The acute and chronic hazard quotients (HQ) were calculated for each pesticide as the ratio of the highest pesticide concentration measured and the non-observed effect concentration (NOEC) for the most sensitive species of each of the three trophic levels evaluated (fish, macroinvertebrates, and algae). Toxicity data were obtained from the Aquatic Information Retrieval of the US Environmental Protection Agency (USEPA 2014). Ratios exceeding the risk criteria (ECOFRAM 1999) were indicative that additional studies were required. Further, both the variation of toxicity among species and the pesticide concentrations over a 3-year period were analyzed by joint probability curves (JPC). JPC describes the probability of exceeding the concentration associated with a particular degree of effect. The concentrations reported as lower than LOD were substitute by half of the LOD value and the mean value between LOD and LOQ was assigned for those reported as trace concentrations. For JCP, the acute toxicity data of each pesticide were obtained from scientific literature (USEPA 2014) from all the aquatic organisms which included algae, worms, molluscs, insects, crustaceans, amphibians, and fish. In the case of more than one toxicity data available for one species, the average was used to develop specie sensitivity distributions (SSDs). Macroinvertebrate attributes (taxon density (individual/m^2), taxon richness, and the Shannon-Weaver diversity index) were compared between the irrigation and drainage channels over two growing seasons.

Statistical analyses

To evaluate differences in concentrations for each pesticide and macroinvertebrate metrics between drainage and irrigation channels, the Kruskall-Wallis analysis was performed (Sokal and Rohlf 1995). Pearson's product-moment correlation coefficient (r) was used to analyze relationships between azinphosmethyl, chlorpyrifos, and carbaryl levels and total density, taxon richness, and the Shannon-Weaver diversity index.

Results and discussion

Water quality analyses

Analysis of the physicochemical conditions in irrigation channel showed water close to neutrality (pH 7.40 ± 0.14), with a conductivity of 474.5 ± 108.12 µS/cm and a dissolved oxygen concentration close to the saturation (9.07 ± 0.65 mg/L). The flow rate in irrigation channel ranged from 37.5 to 50.5 L/s (mean flow 44.83 ± 3.78 L/s). The water quality attributes at the sampling sites in the drainage channels were similar to that in the irrigation channel with similar pH, higher conductivity (556.31 ± 73.13 µS/cm) and lower DO concentrations (7.12 ± 0.41 mg/L). However, the mean flow rate was four times lower (12.45 ± 2.00 L/s) (Table 1).

Since the physicochemical properties among the drainage channels were similar, the results of pesticide concentrations were combined for analyses at each sampling period 2008–2009, 2009–2010, and 2010–2011. The range of concentrations (µg/L) determined for azinphosmethyl, chlorpyrifos, and carbaryl in drainage channels, during the monitoring period, was from non-detected (ND) to 1.02, 1.45, and 11.21 µg/L, respectively. The average concentration distribution for the compounds studied, grouped by sampling year and month, is shown in Fig. 2. The pesticide most frequently detected was chlorpyrifos (61%), followed by azinphosmethyl (44%) and carbaryl (21%). Furthermore, 68, 44, and 18% of the

Table 1 Physicochemical characteristics of irrigation and drainage channels

	Channels	
	Irrigation	Drainage
Water temperature (°C)	14.6–22.2	11.2–20.1
pH	6.8–7.8	6.5–8.2
Conductivity (µS/cm)	244–870	243–943
Oxygen (mg/L)	7.1–10.7	6.7–9.6
Discharge (L/s)	37.5–50.5	7.3–28.1

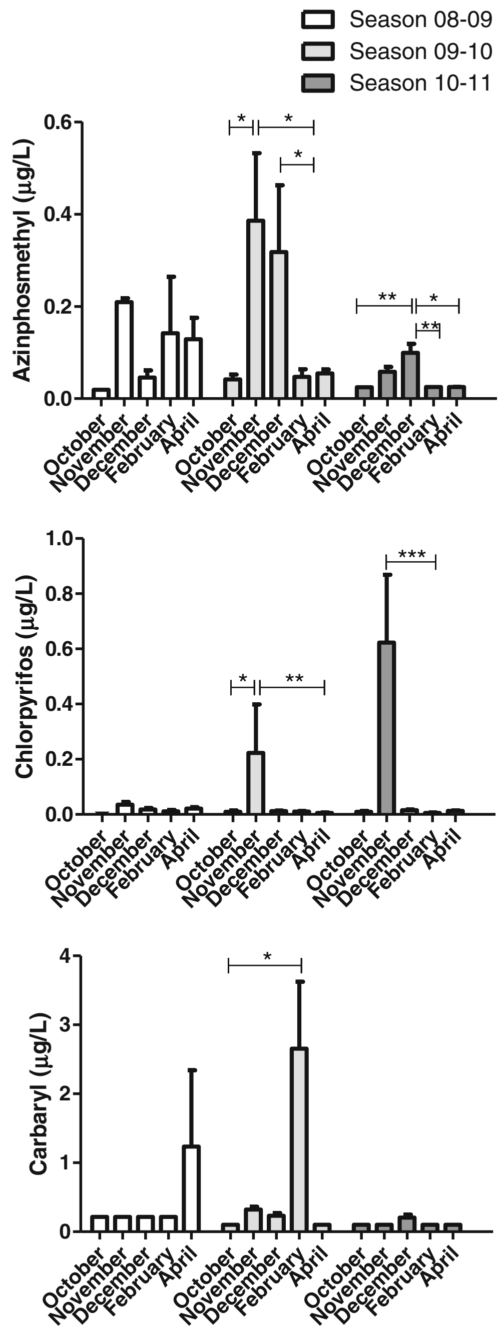

Fig. 2 Concentrations determined for azinphosmethyl, chlorpyrifos, and carbaryl in drainage channels during the sampling seasons (2008–2009, 2009–2010, and 2010–2011). The bars represent the mean (µg/L) + standard error of the mean of each pesticide determination for the drainage channels at each month and sampling period. ***$p < 0.005$, **$p < 0.01$, *$p < 0.05$ by the post hoc Kruskal-Wallis multiple comparison test

samples exhibited one, two, or all three pesticides, respectively. Significantly higher concentrations of chlorpyrifos ($p < 0.05$ and $p < 0.005$, season 2009–2010 and season 2010–2011, respectively) were detected in November in the latter two growing seasons (Fig. 2). Also, significantly higher concentrations of azinphosmethyl were detected during November ($p < 0.05$) and December ($p < 0.05$) in 2009–2010 growing season (Fig.2). In the growing season 2010–2011, significantly higher concentrations of azinphosmethyl were detected during November ($p < 0.05$) (Fig. 2). This region has a semiarid climate with a rainfall annual average of 200 mm with rainfall being negligible during the growing season. This is why spikes in concentrations, e.g., chlorpyrifos, are mainly attributed to agricultural pesticide use patterns. Moreover, November and December are the months were organophosphate pesticides are mostly sprayed. On the other hand, the highest carbaryl concentrations were detected at the end of the pear and apple harvest season, consistent with the carbamate pesticide application period.

Interestingly, non-significant changes in all pesticide concentrations studied were observed in growing season 2008–2009, while in the latter two seasons, there were significant increases in azinphosmethyl, chlorpyrifos, and carbaryl. These results can be due to the agricultural practices and the pesticides utilized in each growing season.

At the irrigation channel (site A), which is not impacted by runoff, only trace concentrations of chlorpyrifos and azinphosmethyl were found at 5 and 2 of the 26 samples, respectively, while carbaryl was not detected (ND). Detections can be attributed to application spray drift, but the residue concentrations were low enough not to impact macroinvertebrate assemblages. The seasonal variation of pesticide concentrations reported here is in accordance with those previously reported in the area (Loewy et al. 2011).

Macroinvertebrate analyses

Forty-three taxa were recorded in the macroinvertebrate assemblages during the study (Table 2). Taxa with greater taxonomic richness were Diptera (ten), Ephemeroptera and Trichoptera (six each one), and Gastropoda (five taxa). Relative abundance of macroinvertebrates in the irrigation channel was mostly represented by Diptera (*Simulium* spp. and Orthocladinae), Amphipoda (*Hyalella curvispina*), and Ephemeroptera (mainly the baetids *Andesiops peruvianus* and *Americabaetis alphus* and the leptophlebiid *Penaphlebia chilensis*). In contrast, in the drainage channels, the most abundant taxa were Amphipoda (*H. curvispina*), Gastropoda (*Heleobia parchapii*, *H. hatcheri*, *Chilina gibbosa*, and *Limnea viator*) and some taxa of Diptera (mainly Chironominae). Particularly in the drainage channels, the density of Baetidae was significantly lower than that in the irrigation channel in November ($p < 0.005$), coinciding with the

Table 2 Mean relative abundance (percentages) of macroinvertebrate taxa in the studied channels of The Río Negro and Neuquén Valley, Patagonia, Argentina

Taxa	Irrigation	Drainage
Turbellaria		
Dugesiidae	1.18	2.47
Oligochaeta		
Naididae	1.94	1.97
Lumbriculidae	3.04	3.21
Hirudinea		
Helobdella sp.	–	0.47
Bivalvia		
Pisidium sp.	0.19	1.68
Gastropoda		
Lymnaea viatrix	0.22	1.16
Physa acuta	–	0.19
Chilina gibbosa	0.56	6.41
Heleobia parchappii	0.91	13.56
Heleobia hatcheri	2.07	1.08
Hydracarina	0.22	0.12
Amphipoda		
Hyalella curvispina	22.90	44.00
Decapoda		
Aegla sp.	0.13	0.06
Collembola	0.65	0.35
Ephemeroptera		
Penaphlebia chilensis	1.29	–
Meridialaris diguilina	0.59	–
Andesiops peruvianus	6.85	–
Camelobaetidius sp.	0.97	0.09
Americabaetis alphus	5.83	2.38
Caenis sp.	0.38	–
Odonata		
Rhionaeschna sp.	–	0.28
Coenagrionidae	–	0.08
Hemiptera		
Belostoma sp.	0.03	0.04
Corixidae	–	0.33
Coleoptera		
Enochrus sp.	0.05	0.04
Liodessus patagonicus	–	0.04
Diptera		
Orthocladinae	12.72	4.27
Chironominae	0.11	12.85
Bezzia sp.	0.19	0.04
Tabanus sp.	0.11	–
Stratiomys sp.	–	0.18
Ephydridae	–	0.30
Empididae	1.13	0.17
Tipula sp.	0.03	0.02
Muscidae	0.13	

Table 2 (continued)

Taxa	Irrigation	Drainage
Simulium sp.	30.70	1.08
Lepidoptera	0.05	0.03
Trichopera		
Brachysetodes major	0.16	–
Smicridea annulicornis	3.52	0.02
Oxyethira bidentata	0.16	0.16
Metrichia neotropicalis	0.54	0.89
Cailloma sp.	0.27	–
Verger sp.	0.19	–

period of pesticide application (during both seasons) (Table 3). Likewise, the density of *Simulium* sp. and Orthocladinae (Chironomidae) were significantly reduced ($p < 0.005$) (Table 3). In contrast, abundance of Gastropoda (during season 2009–2010) and Chironominae (Chironomidae) were significantly higher in drainage channels than the irrigation channel ($p < 0.05$) (Table 3). Finally, the density of the amphipod *H. curvispina* was higher in drainage channels than irrigation channels, but it declined significantly in November in both growing seasons ($p < 0.01$) (Table 3).

Furthermore, taxon richness in drainage channels was always lower than in the irrigation channel (Fig. 3) and it was significantly lower in November ($p < 0.01$). Similar patterns were observed in the Shannon-Weaver diversity index during season 2009–2010 with higher diversity in the irrigation channel than that in the drainage channels, although the differences were only significant in October and February ($p < 0.01$). In the same way, the abundance of macroinvertebrates between channels showed a significant reduction from the November sampling ($p < 0.01$). Moreover, the abundance of macroinvertebrates at the drainage channels was significantly lower in November ($p < 0.01$) than in the other sampling months at the same site (Fig. 3). The decrease in species richness and the abundance observed in November were apparently associated with chlorpyrifos and azinphosmethyl maximum peak concentrations recorded in water during this month. However, correlation analyses indicated that only chlorpyrifos concentrations were negatively correlated with both parameters (the Spearman r^2 −0.61, $p = 0.0051$ and the Spearman r^2 −0.59, $p = 0.0068$ for species richness and abundance correlation, respectively). Several studies showed that taxon richness and macroinvertebrate abundance in freshwater ecosystems decreased as the pesticide concentrations increased (Anderson et al. 2006; Beketov et al. 2013; Liess and von Der Ohe 2005; Schäfer et al. 2007). It is well established that Ephemeroptera are highly sensitive to pesticide pollution (Thiere and Schulz 2004) and particularly to azinphosmethyl (Schulz et al. 2002) and chlorpyrifos (Colville et al. 2008). A field study in Australia (Szöcs et al. 2012) found that Baetidae was among the most pesticide sensitive families. On the other hand, chironomids were the most abundant taxa in streams with high

Table 3 Mean densities (individuals/m2) of macroinvertebrate taxa collected from the irrigation (control) and drainage (impacted) channels in the Río Negro and Neuquén Valley over two growing seasons

	Season 2009–2010						
	October		November		February		Observed
	Control (OC)	Drainage (OD)	Control (NC)	Drainage (ND)	Control (FC)	Drainage (FD)	Relationship[a]
Gastropoda	9.67 ± 4.16	23.33 ± 13.38	14.67 ± 12.66	26.33 ± 10.71	5.67 ± 1.53	52.83 ± 41.37	OD, ND, FD > FC
Hyalellidae	17.00 ± 6.00	89.17 ± 29.88	43.00 ± 11.53	27.00 ± 10.79	49.67 ± 24.44	51.83 ± 27.97	OD > ND,OC
Baetidae	22.00 ± 11.36	4.83 ± 5.67	55.00 ± 25.16	0	51.00 ± 13.45	9.50 ± 7.84	OC, FC, OD > ND
Ortocladinae	38.33 ± 16.26	16.50 ± 4.89	17.33 ± 12.74	2.67 ± 1.63	18.67 ± 8.02	3.17 ± 1.72	OC, FC, OD > ND
Chironominae	2.00 ± 1.00	16.33 ± 11.45	3.00 ± 1.00	23.50 ± 18.01	1.67 ± 1.15	41.00 ± 53.68	OD, ND, FD > FC
Simulidae	62.00 ± 52.05	3.17 ± 5.88	46.67 ± 30.73	2.50 ± 2.07	15.33 ± 14.29	2.50 ± 4.28	OC, NC > OC, ND, FD
	Season 2010–2011						
	October		November		February		Observed
	Control (OC)	Drainage (OD)	Control (NC)	Drainage (ND)	Control (FC)	Drainage (FD)	relationship[a]
Gastropoda	6.33 ± 5.03	42.67 ± 40.33	7 ± 7	36.78 ± 30.27	4.67 ± 5.51	50.83 ± 52.52	n.s.
Hyalellidae	59 ± 15.72	84.33 ± 33.46	71 ± 16	14.56 ± 11.64	44.33 ± 9.45	79.33 ± 51.73	OC, NC, OD, FD > ND
Baetidae	17.33 ± 3.06	6.33 ± 6.89	12.33 ± 7.23	0	11.67 ± 9.71	0.5 ± 0.84	OC, NC > ND, FD
Ortocladinae	43 ± 25.71	21.5 ± 13	8.67 ± 6.35	2.67 ± 1.87	8.33 ± 3.79	5.5 ± 3.45	OC, OD > ND, FD
Chironominae	3.33 ± 1.53	18.33 ± 9.44	4.33 ± 2.31	23.56 ± 15.6	3 ± 1.73	15.5 ± 9.91	OD, ND, FD > OC, FC
Simulidae	120.33 ± 56.96	7 ± 2.37	95 ± 80.73	1.67 ± 4	41.33 ± 71.59	2.50 ± 4.28	OC, NC > ND, FD

n.s. non-significant

[a] Significance of the comparison of the Kruskal-Wallis test ($p < 0.05$)

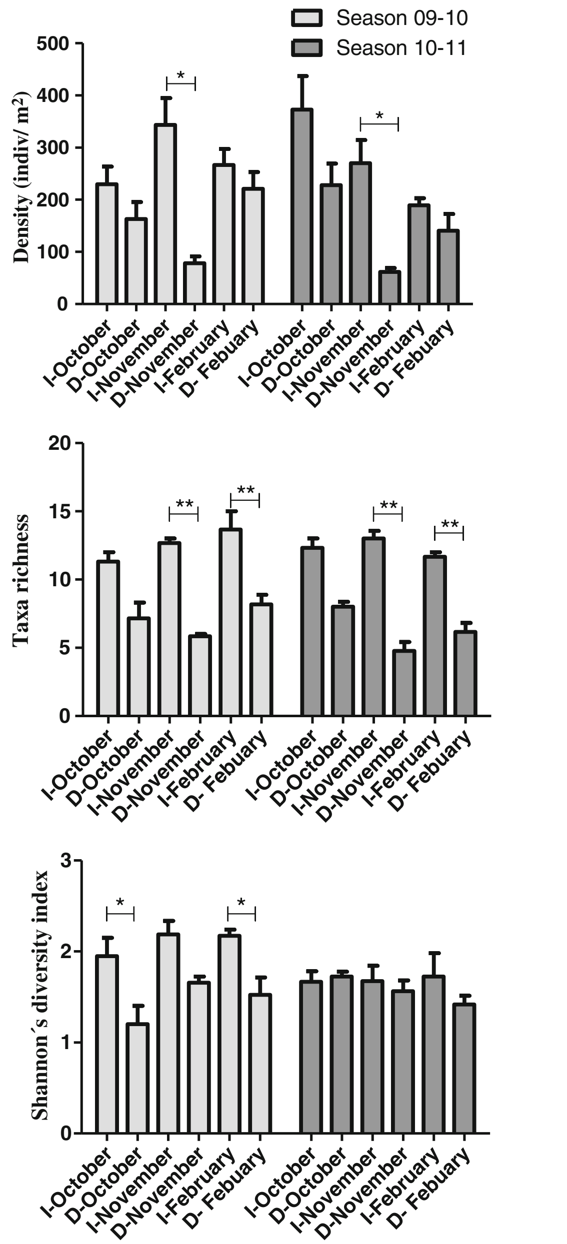

Fig. 3 Density (individual/m2), taxon richness, and the Shannon-Weaver diversity index of macroinvertebrates at the irrigation (*I*) and drainage channels (*D*) during the sampling moths of the production seasons of 2009–2010 and 2010–2011. Bars represent the mean + standard error of the mean; $^{**}p < 0.005$, $^{*}p < 0.05$ by the post hoc Kruskal-Wallis multiple comparison test

detection of pesticides, including chlorpyrifos (Overmyer et al. 2005). Another study showed that Orthocladiinae decrease in number at much lower concentrations than the Chironominae. This finding is probably due to the fact that many species of the latter subfamily live in the sediment whereas the Orthocladiinae usually live in open water (Brock et al. 2000). In contrast, the reduction in densities of Mollusca, Annelida, and Turbellaria was only observed at relatively high exposure concentrations and in a limited number of studies (Crommentuijn et al. 2000; Van Wijngaarden et al. 2005). Moreover, molluscs are among the most tolerant taxa towards organic contaminants (von der Ohe and Liess 2004).

The ultimate aim of ecotoxicology is to determine and predict the effects of contaminants in real-world systems (Newman and Unger 2003). Bioassessment and ecological risk assessment are inherently complementary in nature (King and Richardson 2003). The risk assessments are based on extrapolation from organisms to ecosystems and from small-scale systems to large-scale systems. Routine biomonitoring of aquatic ecosystems generally is performed with the intent of demonstrating a causal relationship between stressors and responses (Clements et al. 2002).

Estimation of the ecological risk assessment by the hazard quotient

According to the calculated acute and chronic HQ (Table 4), the exposure of the three pesticides would not represent a hazard for algae. On the other hand, peak concentrations of azinphosmethyl, chlorpyrifos, and carbaryl might adversely impact on macroinvertebrates. For all three pesticides, the acute HQ exceeded the risk criterion (0.5) for invertebrates. *Palaemonetes pugio* (daggerblade grass shrimp) (Key et al. 2006) was the most sensitive species to azinphosmethyl (HQ = 3.4), while *Paratya australensis* (fresh water shrimp) was the most sensitive species to both chlorpyrifos (HQ = 145.0) and carbaryl (HQ = 7.4). Regarding native macroinvertebrate species, previous acute toxicity assays with the native amphipod *Hyalella curvispina* showed the coexistence of two subpopulations with different susceptibilities to azinphosmethyl (LC_{50} = 390.0 and 1.8 μg/L) (Anguiano et al. 2008). On the other hand, native black fly populations (*Simulium* spp.) might not be severely affected by either azinphosmethyl exposure (LC_{50} = 21 μg/L) (Montagna et al. 2012) or carbaryl (LC_{50} = 18 μg/L) (Montagna et al. 1999). The present study suggested that the susceptible subpopulation might be affected during peak concentration events. Moreover, peak concentrations might be repeated over time due to pest control schemes which may increase the selection pressure for tolerant individuals. In fact, black fly field populations collected from an irrigation channel at this area developed an increased resistance to the pyrethroids fenvalerate (more than 355-fold) and deltamethrin (162-fold) between 1996 and 2008 (Montagna et al. 2012).

Regarding fish, the risk criterion for acute exposure (0.5) was only exceeded by azinphosmethyl (HQ = 1.1). Invertebrates were more susceptible to chronic exposure to azinphosmethyl (HQ = 10.5), chlorpyrifos (HQ = 45.3), and carbaryl (HQ = 15.6) than fish, where the risk criterion (1.0) was only exceeded for azinphosmethyl (HQ = 6.0) (Table 4). Peak concentrations may cause dead of the most susceptible individuals, but also residues that degrade over time may result in chronic and sublethal exposures to non-target

Table 4 Acute and chronic hazard quotient (HQ) from the most susceptible species from representative groups

Pesticide	Acute endpoint[a]	Chronic endpoint[b]	Peak concentration	Acute HQ	Chronic HQ
Azinphosmethyl			1.02		
Algae	10,000.00	NR		1.02^{-4}	–
Invertebrate	0.30	0.097		3.40	10.51
Fish	0.91	0.17[j]		1.12	6.00
Chlorpyrifos			1.45		
Algae	400.00	100.00		3.62^{-3}	0.014
Invertebrate	0.010	0.032		145.00	45.31
Fish	320.00	1.70		1.12	0.85
Carbaryl			11.21		
Algae	50.00	500.00		0.22	0.022
Invertebrate	1.50	0.72		7.47	15.56
Fish	9000.00	445.00		1.24^{-3}	0.025

NR not reported

[a] All values represent NOEC (μg/L) obtained after 48- or 96-h treatment

[b] All values represent NOEC (μg/L) obtained after 6 or 7 days treatment for algae, 21 or more days for aquatic invertebrate or crustacean, and 21 or more days for fish. The peak concentrations are expressed as μg/L. Peak concentrations are expressed as NOEC μg/L. Data were obtained from the Aquatic Information Retrieval of USEPA (USEPA 2014)

organisms (Desneux et al. 2005). Unlike terrestrial organisms, aquatic organisms cannot easily avoid exposure by moving to uncontaminated areas, particularly if pesticides are water soluble. Moreover, pesticides uptake in aquatic invertebrates occurs through respiration (gills and trachea), feeding and through the cuticle (Pisa et al. 2015). Also, chronic and sublethal effects may be linked to reproduction (fertility, fecundity, and sex ratio), development time, longevity, and behavior (de Franca et al. 2017; Desneux et al. 2005; El Hassani et al. 2008). Therefore, sublethal effects could involve several and successive modifications at different biological levels from genes to population.

Since most of the HQ values highly exceeded the risk criterion for macroinvertebrates, a probabilistic risk assessment was next performed.

Probabilistic risk assessment

The effects of the three pesticides on aquatic freshwater organisms were characterized by JPC (Fig. 4). At the left, it has been represented the distribution of pesticide concentrations determined during the growing seasons 2008–2009, 2009–2010, and 2010–1011. At the right, the curves represent the distribution profile of LC_{50} values from all the aquatic

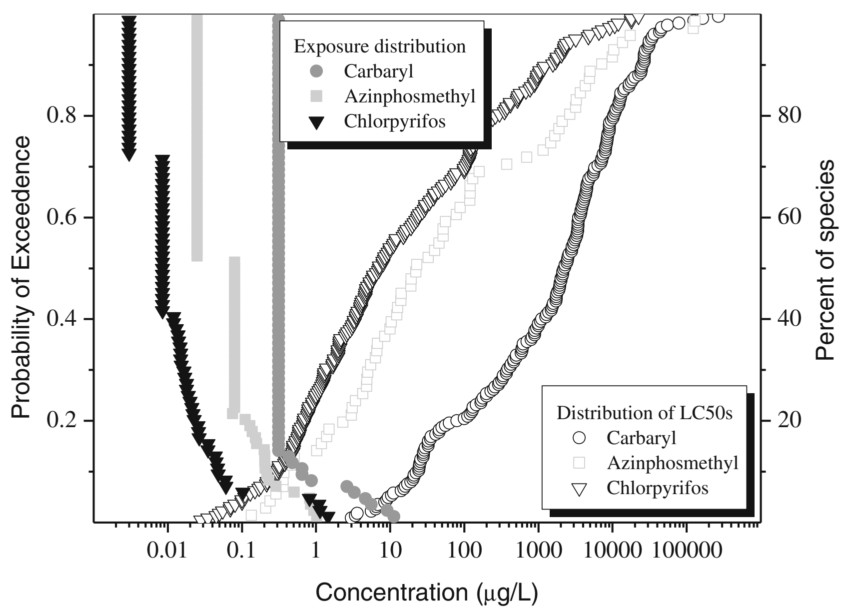

Fig. 4 Concentration exceedence probabilities and toxicity distribution profile for azinphosmethyl, chlorpyrifos, and carbaryl. Exposure distribution is shown for azinphosmethyl (light gray filled squares), chlorpyrifos (black filled triangles), and carbaryl (gray filled circles). Distribution of LC_{50} is shown for azinphosmethyl (empty squares), chlorpyrifos (empty triangles), and carbaryl (empty circles)

organisms which included algae, worms, molluscs, insects, crustaceans, amphibians, and fish obtained from the Aquatic Information Retrieval of the USEPA (USEPA 2014). For each concentration on the *x*-axis, the curve indicates the frequency that a concentration was exceeded during the time period analyzed and the percentage of species affected. The tenth centile of the distribution of acute toxicities to species was used as the assessment endpoint according with the Aquatic Risk Assessment and Mitigation Dialog Group (SETAC 1994). The effects of the three pesticides on aquatic organisms, characterized by JPC, showed that the LC_{50} of 10% of the species were exceeded five and three times the concentrations of azinphosmethyl (0.66 μg/L) and chlorpyrifos (0.31 μg/L) during the analyzed period. Carbaryl concentrations were always below the assessment endpoint.

The results showed low probability of occurrence of adverse effects by exposure of individual insecticides. However, the risk of the mixture could be of greater concern considering that both organophosphates and carbamates are anticholinesterase pesticides. As it was mentioned above, 44 and 18% of the samples exhibited two and three insecticides, respectively. Moreover, in vivo exposures to binary mixtures of organophosphates and carbamate produced additive or synergistic acetylcholinesterase (AChE) inhibition in the brains of juvenile coho salmon (Laetz et al. 2009; Moore and Teed 2013). In addition, synergism has been observed with several organophosphates and pyrethroids insecticides. The synergism was attributed to the inhibition of esterases by the organophosphates, thus reducing the detoxification of pyrethroids (Belden et al. 2007; Deneer 2000).

Conclusions

The results found in this work, obtained from the tiered risk assessment approach integrated with macroinvertebrate biomonitoring demonstrated that during spring-summer, the concentration of different pesticides increased in drainage waters, which flow to the Neuquén River basin. The macroinvertebrate assemblages in the drainage channels were highly affected by pesticides, mainly chlorpyrifos, its concentration being negatively correlated with taxonomic richness and abundance. Macroinvertebrates responded to acute and chronic pesticide contamination by elimination of the more susceptible taxa and enhancing the abundance of both the most tolerant ones and those with higher capabilities of adaptation. The most remarkable changes were the decrease in sensitive taxa such as Baetidae and the increase in tolerant taxa such as Chironomidae and Mollusca. Moreover, the pesticide levels found mainly impacted macroinvertebrate community, as indicated by the hazard quotients and the JCP analysis.

The integration of risk assessment with biomonitoring proved to be complementary approaches for the evaluation and prediction of the effects of pesticide contamination, demonstrating the causal relationship between stressors and the responses of macroinvertebrate communities.

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