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Effects of insecticides on freshwater invertebrate communities of small streams in soy-production regions of South America

By

Elizabeth Shirin Hunt

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University of California, Berkeley

Committee in charge:

Professor Vincent H. Resh, Chair

Professor Stephanie M. Carlson

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Abstract

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Doctor of Philosophy in Environmental Science, Policy, and Management

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Professor Vincent H. Resh, Chair

In this dissertation, I examined the occurrence of insecticides in streams in intensive soy production regions of South America, and their effects on invertebrate communities. Recently soy has become a major export crop in South America, and the insecticides used are highly toxic to aquatic invertebrates. I adapted the Species at Risk pesticide index ($SPEAR_{pesticides}$), which was developed in Europe to assess effects of pesticide contamination in agricultural streams. I then explored the relative importance of insecticides in comparison to other agricultural stressors, and the potential for riparian buffers to mitigate pesticide transport and impacts. My study sites were on small streams adjacent to agricultural fields in four soy production regions: two regions in the Argentine Pampas (La Plata-Magdalena and Arrecifes), and one region each in the Atlantic forest habitat of Brazil and Paraguay.

Commonly used insecticides were detected at high frequencies in all three countries, and pyrethroids insecticides were the most likely to occur at acutely toxic concentrations. Samples with highest toxicity were collected from streams with riparian buffer width less than 20 m, and buffer width was the most important predictor variable in explaining insecticide levels. I evaluated the toxicity of the four most commonly detected insecticides to *Hyalella curvispina*, a freshwater amphipod that is widespread in South America. The lowest LC50 values were found for the pyrethroid insecticides lambda-cyhalothrin and cypermethrin, followed by chlorpyrifos and alpha-endosulfan.

After adapting the $SPEAR_{pesticides}$ index for local invertebrate communities in the Argentina streams, I found that $SPEAR_{pesticides}$ was the only response metric that was significantly correlated with insecticide levels. Multiple regression showed that insecticide toxicity was the most important stressorin explaining variability in the $SPEAR_{pesticide}$ index.

I then evaluated the relative importance of insecticides and other agricultural stressors on invertebrate communities in Atlantic Forest streams. Although buffer widths in Brazil streams were negatively correlated with insecticide concentrations, and had a moderate importance in mitigating effects on some sensitive taxa, insecticides had little importance in explaining variability in invertebrate communities. The forested riparian buffer zones are likely to have mitigated the effects of pesticides on stream invertebrate communities in these regions.

TABLE OF CONTENTS

DEDICATION

Dedicated to Oof and the Ancient One, and to all living beings on this extraordinary planet (especially the weird ones)

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CHAPTER 1

Introduction

Introduction

In recent years, soybean production has become a major export crop for multiple countries in South America, including Brazil, Argentina, Paraguay, Uruguay, and Bolivia. Between 1986 and 2010, the total area in soy production in the Americas increased from 37 to 79 million hectares (Mha), and most of this expansion occurred in Argentina, Brazil, and Paraguay (Garrett et al. 2013). Between 1995 and 2011, soy cultivation area expanded by 126% and 209% in Brazil and Argentina, respectively (Castanheira and Freire 2013). In Paraguay, soy cultivation area increased from 1.3 Mha in 2000-2001 to 2 Mha in 2007-2008 (Garcia-Lopez and Arizpe 2010). Land use changes caused by expansion of soy cultivation in South America have raised a number of environmental concerns, including reductions in ecosystem complexity, loss of biodiversity, deforestation, increased erosion, adverse effects of agrochemicals, and increased greenhouse gas emissions (Botta et al. 2011; Castanheira and Freire 2013; Lathuilliere et al. 2014).

Conversion of land to intensive agriculture can result in degradation of adjacent streams and stream ecosystem through impacts such as nutrient enrichment, sedimentation, pesticides, deforestation (Gücker et al., 2009; Jones et al., 2001; Matthaei et al., 2010). Benthic macroinvertebrate communities have been shown to be adversely impacted by agriculture adjacent to streams through multiple mechanisms. Agriculture-related stressors can include habitat degradation (e.g. loss of cover, deposition of fine sediments), hydrological modification (e.g. channelization, less diversity in pool/run/riffle regimes) and impacts to water quality (e.g. pesticide toxicity, nutrient eutrophication, increased turbidity and conductivity)(Matthaei et al., 2010; Stehle and Schulz, 2015; Stone et al., 2005; Whiles et al., 2000).

Pesticides used in agriculture can have severe impacts on stream water quality and ecosystems. A recent metaanalysis of 838 studies across 73 countries found that over 50% of measured insecticide concentrations in water bodies exceeded regulatory threshold levels for surface waters or sediments (Stehle and Schulz, 2015). Arecent analysis of data from Europe and Australia reported that pesticides reduced both species and family richness of aquatic invertebrate communities (Beketov et al., 2013). The insecticides used in soy production in South America are known to be especially toxic to aquatic invertebrates (Mugni et al., 2011).

A life cycle analysis of the soy-biodiesel crops produced in Argentina for export concluded that the aquatic toxicity impacts from soy-production pesticides were substantially higher than their terrestrial toxicity impacts, with the pyrethroid insecticide cypermethrin being the main contributor (Panichelli et al. 2009). Although application rates of the herbicide glyphosate in the cultivation of genetically modified soy are much higher than those of fungicides and insecticides, the potential toxic impact of glyphosate and other herbicides in aquatic areas near soy production systems of South America are considered to be negligible compared to those of fungicides and insecticides (Nordborg et al. 2014). Insecticide application rates are approximately double those of fungicides, and the insecticides most frequently used in soy production have very high aquatic toxicity (Nordborg et al. 2014).

Stream buffer width may be one of the most important factors in mitigating transport of pesticides, sediment,and other pollutants to streams in agricultural areas (Bunzel et al., 2014; Jones et al., 2001; Rasmussen et al., 2011; Stone et al., 2005), butbuffer zone requirements differ substantially among the three major soy production countries in South America. Riparian buffer zones are required to be maintained in both Brazil and Paraguay, although specific requirements are in flux. For example, Paraguay, requires a protected zone of 100 m around all water bodies.

In Brazil, a new forest code was approved in 2012 (Law No.12.651/12) establishing that riparian buffer zone requirements should vary with the general use of the land adjacent to the water body, the aquatic environment, the stream width, and the size of the rural property. In contrast, in Argentina there are no national requirements for stream buffers.

In this dissertation, I examined the occurrence of insecticides in small streams in intensive soy production regions of South America, and their effects on stream invertebrate communities. I adapted the Species at Risk (SPEAR) pesticide bioassessment index ($SPEAR_{pesticides}$), which was developed in Europe to assess effects of pesticide contamination in agricultural streams (Liess and Von der Ohe, 2005), for my study region. In addition, I explored the relative importance of insecticide toxicity effects in comparison to other agricultural stressors on invertebrates, and the potential for riparian buffers to mitigate pesticide transport and other adverse effects on stream.

Study regions

The study sites were located on small streams that flowed through agricultural fields in four soy production regions: two regions in the Argentina Pampas (La Plata-Magdalena and Arrecifes), and one region each in the former Atlantic forest habitat of Brazil and Paraguay (Figure 1). In the La Plata-Magdalena region, the principal land use was cattle grazing, with scattered plots of soy production and other agriculture. In the three other regions, intensive soy production was the predominant land use. In the La Plata-Magdalena region, five streams were sampled during five monitoring events in the 2011 to 2012 season only, including three sampling sites in one watershed and the remaining sites in separate watersheds. In the Arrecifes region, 16 sites were sampled over three years (2012-2014), and all sampling sites were on tributaries of the Arrecifes River. In Paraguay, 17 sites were sampled over two seasons (January and December 2013), and all sampling sites were on tributaries of the Pirapó River in the state of Itapúa. In Brazil, 18 sites were sampled once in November 2013, and all sampling sites were on tributaries of the San Francisco River in the state of Paraná. All study watersheds were tributaries of the Paraná/La Plata River.

Chapter Overview

In Chapter 2, I describe the results of my investigations on concentrations and detection frequencies of insecticides in the four study regions. I also conducted a regression analysis to evaluate the influence of riparian buffer width on insecticide concentrations in streams.

In Chapter 3, I evaluated the toxicity of the four most commonly detected insecticides (cypermethrin, lambda-cyhalothrin, chlorpyrifos and endosulfan) to *Hyalella curvispina*, a freshwater amphipod that is widespread in South America and is closely related to *H. azteca*, a standard test species in the United States. For each of these insecticides in both sediment and water, I determined median lethal concentration (LC50) values for *H. curvispina*. I then calculated species sensitivity distributions (SSDs) for freshwater invertebrate taxa using results of my study and other available data.

In Chapter 4, I investigated relationships among insecticide concentrations and aquatic invertebrate communities in 22 streams of two soy production regions of the Argentine Pampas over three growing seasons. Along with standard macroinvertebrate bioassessment metrics, I applied the $SPEAR_{pesticides}$ index to evaluate relationships between sediment insecticide toxic units (TUs) and invertebrate communities associated with both benthic habitats and emergent vegetation. I then performed a multiple regression analysis to evaluate the influence of multiple agricultural stressors and habitat variables.

In Chapter 5, I evaluatedthe influence and relative importance of insecticides and other agricultural stressors in determining variability in invertebrate communities in small streams in intensive soy production regions of Brazil and Paraguay. The riparian buffer zones in these regions generally contained native Atlantic forest remnants and/or introduced tree species at various stages of growth, and I evaluated the effectiveness of the riparian buffer in mitigating adverse effects of soy production on streams.

In Chapter 6, I summarize the overall conclusions and important findings of my dissertation research. I also lay outsome possible explanations for why the findings for insecticide effects on invertebrate communities differed between the Argentina Pampas streams and the Atlantic Forest streams. I then discuss future research and management needs in the region.

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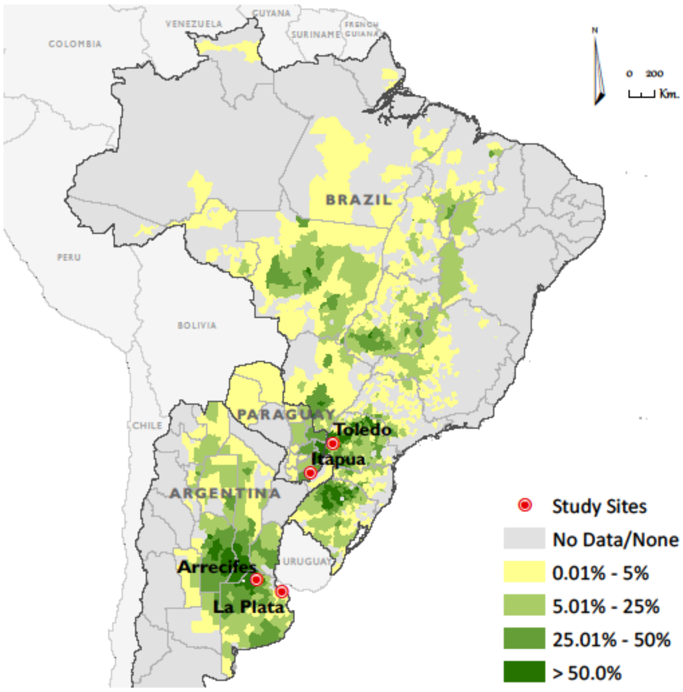

Figure 1. Study regions and soy production intensity as percent of total land use by province or department in Argentina, Brazil, and Paraguaybased on data reported by governments (Argentina: http://www.minagri.gob.ar; Brazil: http://www.ibge.gov.br; Paraguay: http://www.mag.gov.py)

CHAPTER 2

Insecticide concentrations in stream sediments of soy production regions of South America

Insecticide concentrations in stream sediments of soy production regions of South America

Abstract

Concentrations of 17 insecticides were measured in sediments collected from 53 streams in soy production regions of South America (Argentina in 2011-2014, Paraguay and Brazil in 2013) during peak application periods. Although environmental regulations are quite different in each country, commonly used insecticides were detected at high frequencies in all regions. Maximum concentrations (and detection frequencies) for each sampling event ranged from: 1.2–7.4 ng/g dw chlorpyrifos (56-100%); 0.9–8.3 ng/g dw cypermethrin (20-100%); 0.42–16.6 ng/g dw lambda-cyhalothrin (60-100%); and 0.49–2.1 ng/g dw endosulfan (13-100%). Other pyrethroids were detected less frequently. Banned organochlorines were most frequently detected in Brazil. In all countries, cypermethrin and/or lambda-cyhalothrin toxic units (TUs), based on *Hyalella azteca* LC50 bioassays,were occasionally >0.5 (indicating likely acute toxicity), while TUs for other insecticides were <0.5. All samples with total insecticide TU> 1 were collected from streams with riparian buffer width<20m. A multiple regression analysis that included five landscape and habitat predictor variables for the Brazilian streams examined indicated that buffer width was the most important predictor variable in explaining total insecticide TU values. While Brazil and Paraguay require forested stream buffers, there were no such regulations in the Argentine Pampas, where buffer widths were smaller. Multiple insecticides were found in almost all stream sediment samples in intensive soy production regions, with pyrethroids most often occurring at acutely toxic concentrations, and the greatest potential for insecticide toxicity occurring in streams with minimum buffer width < 20m.

Introduction

In recent years, soybean production has become a major export crop for multiple countries in South America, including Brazil, Argentina, Paraguay, Uruguay, and Bolivia. Between 1986 and 2010, the total area in soy production in the Americas increased from 37 to 79 million hectares (Mha), and most of this expansion occurred in Argentina, Brazil, and Paraguay (Garrett et al. 2013). Between 1995 and 2011, soy cultivation area expanded by 126% and 209% in Brazil and Argentina, respectively (Castanheira and Freire 2013). In Paraguay, soy cultivation area increased from 1.3 Mha in 2000-2001 to 2 Mha in 2007-2008 (Garcia-Lopez and Arizpe 2010). Land use changes caused by expansion of soy cultivation in South America have raised a number of environmental concerns, including reductions in ecosystem complexity, loss of biodiversity, deforestation, increased erosion, adverse effects of agrochemicals, and increased greenhouse gas emissions (Botta et al. 2011; Castanheira and Freire 2013; Lathuilliere et al. 2014).

A life cycle analysis of the soy-biodiesel crops produced in Argentina for export concluded that the aquatic toxicity impacts from soy-production pesticides were substantially higher than their terrestrial toxicity impacts, with the pyrethroid insecticide cypermethrin being the main contributor (Panichelli et al. 2009). Although application rates of the herbicide glyphosate in the cultivation of genetically modified soy are much higher than those of fungicides and insecticides, the potential toxic impact of glyphosate and other herbicides in aquatic areas near soy production systems of South America are considered to be negligible compared to those of fungicides and insecticides (Nordborg et al. 2014). Insecticide application rates are approximately double those of fungicides, and the insecticides most frequently used in soy production have very high aquatic toxicity (Nordborg et al. 2014).

Insecticides are typically applied several times to each soy crop, and are used primarily to control lepidopteran pests during plant growth, and hemipteran pests during the fruiting stage. Lepidopteran pests are often controlled by applications of chlorpyrifos, an organophosphate, and hemipteran pests by endosulfan, an organochlorine. Pyrethroids, especially cypermethrin, are commonly used for both types of pests, and are often applied at the same time as other pesticides (Di Marzio et al. 2010; OPDS 2013). In Brazil, diamides and growth inhibitors are becoming more frequently used to control lepidopteran pests, while mixtures of neonicotinoid and pyrethroid insecticides are often used to control hemipteran pests. Contrary to recommendations from pest control advisors, pesticide applications for soy production in Brazil are primarily done prophylactically, with four to six applications per year (Bueno et al. 2011). The same trend is true in Argentina, with cypermethrin often being added to herbicide applications in order to prevent lepidopteran pests from laying eggs (OPDS 2013). Moreover, the systemic neonicotinoid insecticide imidacloprid is commonly used in Paraguay and Brazil as a seed treatment, and is also applied as a spray later in the season along with pyrethroids, such as lambda-cyhalothrin or cypermethrin.

Multiple studies have detected soy production insecticides in both sediment and water collected from streams in Argentina and Brazil; however, most studies did not include all of the most frequently used insecticides, and data were not always comparable because of the use of variable matrices, methods, and reporting limits (Jergentz et al. 2004a; Mugni et al. 2010; Di Marzio et al. 2010; Marino and Ronco 2005; Possavatz et al. 2014; Casara et al. 2012; Miranda et al. 2008; Laabs et al. 2002). Several studies in Argentina and Brazil have found associations between stream insecticide concentrations and effects to aquatic invertebrates and/or fish (Jergentz et al. 2004a; Rico et al. 2010; Di Marzio et al. 2010; Mugni et al. 2010; Chelinho et al 2012); however, no studies of this type have been published on data collected from Paraguay.

Stream buffer width may be one of the most important factors in mitigating transport of pesticides to streams in agricultural areas (Bunzel et al. 2014; Rasmussen et al. 2011), but buffer zone requirements differ substantially among the three countries included in the present study. Riparian buffer zones are required to be maintained in both Brazil and Paraguay, although specific requirements are in flux. For example, in Paraguay, Resolution 485/03 by the Ministry of Agriculture requires a protected zone of 100 m around all water bodies. In Brazil, a new forest code was approved in 2012 (Law No.12.651/12) establishing that riparian buffer zone requirements should vary with the general use of the land adjacent to the water body, the aquatic environment, the stream width, and the size of the rural property. As a general rule for stream widths of 10m or less, the legislation requires a buffer width of 15m of native riparian forest in rural areas or 30m if in areas newly converted for rural activities. In contrast, in Argentina there are no national requirements for stream buffers. Moreover, stream buffer zones in the Argentine Pampas are generally unregulated, and many small streams in the most intensive soy production regions of the Santa Fe and Cordoba provinces are completely channelized with crops planted right up to the banks (no buffer zones). Some Argentine provinces do prohibit pesticide application within a specific distance from surface water (Chaco: Law 7032 – DR 1567/13; Formosa: Law 1163 – DR 109/02; Río Negro: Law 2175 – DR 769/94).

The objectives of the present study were to: (1) measure and compare insecticide concentrations in sediments collected from streams in four soy production regions: two in the Pampas of Argentina, one in eastern Paraguay, and one in south Brazil; (2) evaluate the potential for acute toxicity of insecticides on sensitive aquatic invertebrate taxa, such as *Hyalella* spp.; and, (3) evaluate the relationship between buffer strip widths and insecticide concentrations in stream sediments, taking into account the influence of other environmental variables.

Methods

Study Locations and Sampling Schedule

The study sites included small streams that flowed through agricultural fields in four soy production regions: two regions in the Argentina Pampas (La Plata-Magdalena and Arrecifes), and one region each in the former Atlantic forest habitat of Brazil and Paraguay (Figure 1). In the La Plata-Magdalena region, the principal land use was cattle grazing, with scattered plots of soy production and other agriculture. In the three other regions, intensive soy production was the predominant land use. In the La Plata-Magdalena region, five streams were sampled during five monitoring events in the 2011 to 2012 season only, including three sampling sites in one watershed and the remaining sites were located in separate watersheds. In the Arrecifes region, 16 sites were sampled over three years (2012-2014), and all sampling sites were on tributaries of the Arrecifes River. In Paraguay, 17 sites were sampled over two seasons (January and December 2013), and all sampling sites were on tributaries of the Pirapó River in the state of Itapúa. In Brazil, 18 sites were sampled once in November 2013, and all sampling sites were on tributaries of the San Francisco River in the state of Paraná. All study watersheds were tributaries of the Paraná/La Plata River.

Streams selected for the present study were not channelized, and most had a buffer strip of at least 5 m from the crops (Tables S1, S2). In the Brazil and Paraguay streams, the buffer zones generally contained Atlantic forest remnants and/or introduced tree species. In both Argentina regions, the buffers generally contained grasses and low shrubs with occasional trees. Minimum buffer widths were measured immediately upstream of sampling sites, and confirmed with LANDSAT images in Brazil and Paraguay. However, confirmation with LANDSAT images was not possible in Argentina, because there generally were not forested areas around streams and it was difficult to differentiate herbaceous vegetation from cropland. Catchments were delineated using topographical maps to estimate catchment size, and in Brazil and Paraguay the percent forest and percent agriculture within each catchment were estimated using LANDSAT images. Substrates in streams of both Argentina regions generally consisted of sediment with no rocks and little woody debris, although a few sites in Arrecifes contained some gravel. Substrates in Brazil and Paraguay streams usually contained relatively large amounts of rocks and/or cobble, and tended to have higher gradients and faster velocities than streams in Argentina. Stream depths ranged from about 0.6 m to > 2 m (although all except two in the La Plata region were < 1 m), and widths ranged from about 3 m to about 25 m (Table S2). While streams in Brazil and Paraguay were generally free of aquatic vegetation, most streams in Argentina included emergent vegetation (e.g.*Typha* spp. and *Scirpus* spp.) and submerged vegetation (e.g. *Potamogeton*, *Ceratophyllum* and *Egeria*), and many in the La Plata-Magdalena region were also characterized by abundant floating vegetation (e.g.*Eichornia*, *Lemna* and *Azolla*).

Stream sampling was timed to coincide with peak insecticide application periods, which varied by region depending on planting time. Soy can either be planted as an early season crop or a late season crop. In the Argentine Pampas, the early season crop was planted in October or November and harvested in February, while in Paraguay and southern Brazil it was planted in September or October and harvested in January. The late season crop was typically planted between December and February and harvested several months later. In the Argentine Pampas, peak insecticide applications for soy production usually occurred in late December to early February, while in Paraguay and southern Brazil they occurred in November and December.

Field water quality measurements

At each sampling site, pH, conductivity, dissolved oxygen, and temperature were measured with a Yellow Springs Instruments SI 556 multi-parameter probe (Yellow Springs, OH, USA). Turbidity was measured with a portable turbidity meter (Hanna Instruments 93414, Woonsocket, RI, USA), and maximum and average water velocities were measured with a current meter (Global Water FP311, College Station, TX, USA).

Sample collection

Based on the properties of the insecticides analyzed, streambed sediments rather than water samples were examined. Most insecticides commonly used in soy production in South America have low water solubility, and a high affinity to bind to soil and sediments based on chemical properties, such as k_{oc} (Tables 1 and 2). Moreover, pesticide concentrations in stream water often occur as ephemeral events, and peak immediately following the first rain after application (Schäfer et al. 2011). However, elevated concentrations of the target insecticides can persist longer when they are associated with sediments (Jergentz et al. 2005). In all of the regions studied, precipitation occurs often during the peak pesticide application period. Sampling events in the present study were generally timed to occur within a week after a heavy rainfall during the peak insecticide application season.

Sediment samples were collected with a stainless steel scoop from the top two centimeters, generally from depositional areas depending on depth, access, and availability of sediment. Composite samples were prepared from 3 to 5 locations at each site and placed in pesticide-free amber glass jars with Teflon lids, which were kept in coolers on ice until arrival at the laboratory where they were kept refrigerated until extraction (maximum of 5 d), or frozen for later extraction (maximum of 4 mo). After thoroughly homogenizing each sample in the laboratory, an aliquot was taken from each sample for analysis of total organic carbon by ferrous sulfate titration (USDA 1996). A separate sample was collected at each location for sediment grain size analysis (Table S2).

Chemicals

All pesticide standards, internal standards (lindane d6 and chlorpyrifos d10), and the surrogate standard decachlorobiphenyl (DCBP) were purchased from Accustandard and had purities > 93% as reported by Accustandard (New Haven, CT, USA). The solvents used in extractions and analysis were all pesticide grade. Granular copper used in sample extractions was purified by covering with methylene chloride, shaken vigorously, and allowed to dry in the hood for 24 h. During the first 18 months of the project, gas chromatography coupled with electron capture detection (GC-ECD) was used to analyze the insecticides reported to be most frequently used in Argentina on soy crops including cypermethrin, chlorpyrifos, lambda-cyhalothrin, and endosulfan (Table 1).

Throughout the project, information on pesticide use was obtained by interviewing personnel from government agencies, universities, pesticide manufacturers, and grower cooperatives in all three countries studied, and by searching documents from all sources including grey literature. In 2013 and 2014, analysis of organochlorine pesticides was added, because of concerns about their potential illegal application (Table 2). For quantification of the larger analyte list, the more advanced method of a GC coupled with a mass spectrometer (GC-MS) was used. Analysis of additional pyrethroids and the synergist piperonyl butoxide (PBO) was also added when the new method was implemented (Table 2). Although PBO is not present in insecticide formulations sold for use in soy production, it is possible that growers are mixing it with pyrethroid pesticides to increase their efficacy, or it may come from other sources such as tick control in farm animal production.

Extraction procedure

Extraction procedures followed You et al. (2004b), who demonstrated that sonication provided good recovery for the pesticides of interest (You et al. 2004b; You and Lydy 2007; You et al. 2008). After each sample was thoroughly homogenized manually, approximately 20 g of sediment (wet weight) was removed, spiked with 100 ng of thesurrogate DCBP, and mixed with 4 g of copper and anhydrous Na_2SO_4 in an ice-cooled beaker until the sediment was sufficiently dry. A 50-ml aliquot of a 50:50 mixture of acetone and methylenechloride was added, and the mixture was sonicated for 5 minutes in 3-s pulse mode using a high-intensity ultrasonic processor at an amplitude of 60 (model VCX 500; Sonics and Materials, Newtown, CT, USA). The extract was decanted and filtered through a Whatman no. 41 filter paper (Whatman, Maidstone, UK) filled with approximately 2 g of anhydrous Na_2SO_4. This procedure was repeated two additional times with a sonication time of 5 minutes each time. Extracts were combined and decreased to approximately 1- 2 ml by evaporation.

Cleanup of extracts

Prior to cleanup, extracts for the methylene chloride andacetone:methylene chloride mixture were solvent-exchanged to hexane, and the volumes of all treatments were reduced to 0.5 to 1ml under nitrogen gas. A Envi-Carb II/primary - secondary amine solid phase extraction (SPE) cartridge was connected to a vacuum manifold, adding 1 g of purified sodium sulfate to the top of the sorbent to remove any residual water, then primed with 3 ml of hexane.The extract was then loaded onto the cartridge. Next, 7 ml of a 30:70 methylene chloride/hexane mixture was added to the cartridge, the extract was removed from the vacuum manifold and reduced to a volume of 0.5 to 1 ml under nitrogen gas. The collection vial was then rinsed three times with 0.5 ml of a 0.1% acetic acid in hexane solution and added to the GC vial. The volume was further reduced to 1 ml for analysis. The acidification step was used to minimize isomerization of the pyrethroids (You and Lydy 2007). Granular copper was added to extracts and placed on a shaker (Lab Rotator model G-2, New Brunswick Scientific Co., NJ, USA) for 2 to 3 h when high residual sulfur was detected in the extracts. Once at final volume, internal standards were added at a concentration of 20 ng/ml (for GC/MS analysis only) and the samples were stored at -20°C until analysis.

Analytical methods

Gas Chromatograph-Electron Capture Detector

During the 2011 to early 2013 sampling period, analysis of the most commonly used insecticides (Table 1) was performed on an Agilent 6890 series GC equipped with an Agilent7683 autosampler and a micro- ECD (Agilent Technologies, Palo Alto, CA, USA). Two columns - a HP-5MS (30 m x0.25 mm x0.25 μm film thickness; Agilent) and a DB-608 (30 m x0.25 mm x0.25 μm film thickness; Agilent) were used to confirm the analytical results. Helium and nitrogen were used as the carrier and makeup gas, respectively. A 2 µl sample was injected into the GC using a pulsed split-less mode. For the DB-608, the oven was set at 100°C, heated first to 250°C at 10°C/min increments, then to 280°C at 3°C/min increments and finally held at 280°C for 23 minutes. For the HP-5, the oven was set at 100°C, heated to 190°C at 5°C/min increments, then to 214°C at 6°C/min increments, then to 280°C at 6°C/min increments and finally held at 280°C for 20 minutes. The flow rates of carrier gas were 1.7 ml/min and 2.0 ml/min for the HP-5MS and DB-608 columns, respectively. Calibration was based on area using three to six external standards. The standard solutions were made by dissolving 2.5,10, 50, 100, or 250 µg/L of each pesticide and surrogate in hexane. The calibration curves generated were linear within this concentration range. Qualitative identity was established using a retention window of 1% with confirmation on a second column, and quantitation was performed using external standard calibration.

Gas chromatography - mass spectrometry

For the 2013 to 2014 sampling period, a longer analyte list was used, and quantification of the samples was completed on an Agilent 6850 gas chromatograph with a 5975 XL mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Piperonyl butoxide was quantified in electron impact (EI) mode, while all of the other target pesticides were quantified in negative chemical ionization (NCI) mode. The analytes were separated for both EI and NCI modes on a HP-5MS column (30 m x 0.25 mm, 0.25µm film thickness, Agilent Technologies) initially set at 50°C, and heated to 295°C at 10°C/min. Inlet, ion source, and quadrupole temperatures were 260, 230, and 150°C, respectively. A 2.0 µl sample was injected in pulsed splitless mode at 7.59 psi. Helium was the carrier gas and column flow was 1.0 ml/min. Identification of the target pesticides was based on detecting the target and qualifier ions (Table S3) within a retention time window of 1%, and the target pesticides were detected in selected ion monitoring (SIM) mode. Quantification was performed using internal standard calibration.

Quality assurance- quality control

A matrix spike (MS), matrix spike duplicate (MSD), and laboratory blank were extracted for at least 5% of the samples. A surrogate (DCBP) was added to each sample prior to extraction to verify the performance of the extraction and cleanup processes. Calibration curves were constructed using six levels for each pesticide and surrogate, while the internal standards (for the GC-MS analyses) were kept constant for all levels at a concentration of 20 ng/ml. Quantitation limits (QL) were based on the lowest calibration standard. Each QL was at least three times the method detection limits calculated measuring a low level spike in clean sediment. The QLs are reported instead of the method detection limits to ensure that low sample concentrations are quantitatively accurate. Sample results were considered to meet quality control criteria if the surrogate recovery was between 50-150%, MS/MSD recovery for each analyte was between 50-150%, no pesticides were detected above QLs in the laboratory blank, and the relative percent differences in MS/MSDs did not exceed 25%. Exceptions to the quality control criteria were identified for each sample (Tables 1 and 2).

Toxic unit calculation

Toxic units (TUs) were calculated for all sediment samples. A TU was equal to the sediment concentration normalized to total organic carbon (TOC), divided by the organism 10-d median level lethal concentration (LC50) for each pesticide. The LC50 values for freshwater aquatic invertebrates were identified from the literature for sensitive species (Table 3). Most of the LC50 values used in the present study were for the amphipod *Hyalella azteca*, which is known to be very sensitive to pyrethroids and chlorpyrifos (Weston and Lydy 2010). Although *H. azteca* does not occur in South America, several closely related species (*H. curvispina*, *H. pampeana*, and *H. pseudoazteca*) are important components of the aquatic invertebrate communities in the region; however, published sediment LC50 values are not available for native species. For endosulfan, the LC50 for the more sensitive *Chironomus tentans* was used to calculate TUs, because it is substantially lower than the LC50 for *H. azteca* (You et al. 2004a).Toxicity of pesticides in sediment is highly dependent on organic carbon content; therefore, the concentrations were normalized for total organic carbon to calculate TU values.

Statistical analysis

To evaluate the relationship between buffer width and pesticide concentrations after accounting for other landscape and habitat predictor variables, a linear multiple regression analysis was conducted for the Brazil data set, which had the largest number of sampling sites (18). Insufficient data were available to conduct a similar analysis for Argentina, as minimum buffer widths could not be verified with LANDSAT data and the sample size was small (12 sites). The Paraguay data set did not have sufficient variation in buffer widths to run a regression analysis because 8 of the 17 sites had a minimum buffer width of 100 m (the minimum required by law). The following predictor variables were considered based on their potential to affect pesticide concentrations in stream sediments: minimum upstream buffer width; percent fines (clay and silt fraction) in sediment; percent organic carbon in sediment; stream gradient (slope measured upstream of the sampling site); and, catchment size. Collinearity of these variables was evaluated by examining pair-wise plots, correlation matrices, and variance inflation factors, and variables with the highest multi-collinearity were eliminated. For the linear regression model (lm function in R), predictor variables were square root transformed and the outcome variable (total insecticide TU) was log transformed. A stepwise process was then performed to select final model variables by comparing the Akaike information criterion (AIC) values, using the R function "step". The lmg metric in the relaimpo (Relative Importance for Linear Regression) package was used to evaluate the relative contribution, or variance explained by each predictor variable (Grömping 2006). All statistical analysis was performed with R 3.2.0 (R Development Core Team 2015).

Results and Discussion

Distribution and seasonality of insecticides

Insecticide concentrations and detection frequencies

The most commonly detected insecticides in the three intensive soy production regions were those reported to be the most heavily used: chlorpyrifos, endosulfan (and its degradation product endosulfan sulfate), cypermethrin, and lambda-cyhalothrin (Table 1). Other pyrethroid and organochlorine insecticides were detected occasionally (Table 2).

Chlorpyrifos had the highest detection frequency in allregions examined, and for almost all sampling events (57 to 100% detection frequency, with 29 to 100% above the highest QL of 0.5 ng/g dw). Maximum concentrations ranged from 1.24 to 7.41 ng/g dw, with the highest concentration measured in the La Plata region, which included a mix of agricultural crops and grazing lands. Chlorpyrifos, which is used for a wide variety of crops in Argentina (OPDS 2013) was the only insecticide that was consistently detected in this region; however, this region was studied for only the first season (Dec 2011 – April 2012) and only the four insecticides most commonly used in soy production were measured (Table 1).

Endosulfan and its degradate endosulfan sulfate were frequently detected in all three intensive soy production regions (43 to 100% detection frequency, with 0 to 100% above the highest QL of 0.5 ng/g dw), but less frequently in the mixed use La Plata region (0 – 29%). While the highest concentrations of endosulfan (31.88 ng/g dw), endosulfan sulfate (155.5 ng/g dw) were detected in the La Plata region, it was likely that upstream vegetable greenhouse production contributed to the elevated levels of these compounds, as they were found in spring at the start of the soy planting season. At the time of sampling, endosulfan was commonly applied on many crops in Argentina (OPDS 2013). Maximum endosulfan concentrations in the three intensive soy regions ranged from 0.25 to 4.42 ng/g dw. Although endosulfan was widely used in soy production in all three countries at the start of the present study, it has since been prohibited (UNEP 2011). Although the detection frequencies of endosulfan increased in the latter half of sampling rounds, this was most likely because the analytical method changed from GC-ECD to GC/MS-NCI. When we examined frequency of detection above the higher QL of 0.5 ng/g dw, across all sampling events using either method, the frequency of detections above this threshold decreased in later sampling events (Table 1).

Seven pyrethroids were detected in all three intensive soy production regions, with cypermethrin and lambda-cyhalothrin consistently being the most frequently detected insecticides (Tables 1 and 2). Cypermethrin and lambda-cyhalothrin were detected at similar frequencies in the three intensive soy production regions, and at similar frequencies for each sampling event, ranging from 29 to 100% for both insecticides (0 to 44% above the highest QL of 0.5 ng/g dw). Although the detection frequencies of these two pyrethroids increased in the latter half of the sampling rounds, the frequency of detection above 0.5 ng/g dw remained similar across years. Maximum concentrations ranged from 0.89 to 8.32 ng/g dw for cypermethrin, and 0.42 to 16.57 ng/g dw for lambda-cyhalothrin. The pyrethroids bifenthrin, cyfluthrin, esfenvalerate, deltamethrin, and permethrin were occasionally detected at lower concentrations in all three intensive soy production regions (they were not measured in the La Plata region). Tefluthrin was the only pyrethroid analyzed that was not detected during the project. The pyrethroid synergist PBO was detected frequently in the three intensive soy production regions (8 to 92% of samples), with maximum concentrations from 1.23 to 11.14 ng/g dw.

Dichlorodiphenyltrichloroethane (DDT) was the only prohibited insecticide that was detected frequently. DDT and its degradates DDE and DDD were detected in all three intensive soy production regions, but most frequently in Brazil (100% detection frequency for DDT and DDE, with maximum concentrations of 1.06 and 2.53 ng/g dw, respectively). In the Arrecifes region, the ratio of DDD to DDT was high (4 to 15.1) and DDE was not detected. DDD is most likely to occur under anaerobic conditions, which would be expected in the region because of the low gradient and little riparian cover (Table S2). Other prohibited organochlorinated insecticides that were detected rarely (and usually at or slightly below QLs) included endrin, chlordane, aldrin, and heptachlor epoxide. Banned organochlorinated insecticidesthat were analyzed, but not detected, included lindane, heptachlor, and dieldrin.

Seasonality and timing

A review of studies conducted within the Arrecifes region of Argentina showed that measured concentrations in sediments were highly dependent on the timing of sampling after pesticide applications. For example, the highest concentrations of endosulfan in the soy production regions in the Argentine Pampas were found by Di Marzio et al. (2010), who sampled within 24 h after aerial pesticide application (maximum concentration of 553 ng/g dw in sediment, compared to a maximum of 4.4 ng/g dw for sites in the same regions sampled during the present study). Marino and Ronco (2005) also studied streams in the Arrecifes watershed and reported higher concentrations of cypermethrin (maximum concentration of 1,075 ng/g dw and a mean of 160 ng/g dw) than detected in other studies at the same sites during the same years. Jergentz et al. (2005) measured only 4.4 ng/g dw in suspended sediment collected at the same locations during the same month (Dec 2003), and did not detect cypermethrin in bed sediment samples collected twice the following month. Previous studies in the Arrecifes region by Jergentz et al. (2004a; 2004b) analyzed cypermethrin, chlorpyrifos, and endosulfan in suspended sediment, and only chlorpyrifos and endosulfan were detected in streams samples, although all three pesticides were detected in field runoff samples. Although the present study targeted sampling during peak insecticide application periods, the sampling events may not have captured the highest concentrations occurring immediately after insecticide application and rainfall.

Several other studies in Argentina detected insecticides in water bodies even though they did not sample during the peak soy production season (Bonansea et al. 2013; Agostini et al. 2013; De Geronimo et al. 2014). Regardless, insecticides were detected in all three studies, and Bonansea et al. (2013) found a maximum concentration of cypermethrin of 112.4 ng/L in stream water, which is one of the highest reported detections reported during any season. Although all of these studies included soy production regions, other crops, such as wheat, were grown in soy regions during other seasons, so insecticides may have been applied to control pests in multiple crops.

Comparison to previous studies

The types of insecticides most frequently detected in the present study were generally similar to those detected in most previous studies in the region. In Argentina, most studies on soy production insecticides focused on the Arrecifes region, where they have detected endosulfan (Di Marzio et al. 2010; Jergentz et al. 2004a and 2004b), cypermethrin (Marino and Ronco 2005; Jergentz et al. 2005), and chlorpyrifos (Jergentz et al. 2004a; 2004b). None of these studies analyzed lambda-cyhalothrin. In Brazil, studies have primarily focused on the Mato Grosso state and the Pantanal region, where endosulfan, chlorpyrifos, and lambda-cyhalothrin were detected (Possavatz et al. 2014; Casara et al. 2012; Miranda et al. 2008; Laabs et al. 2002).

Although the neonicotinoid insecticides were not analyzed as part of the present study because there was little evidence of their use at the start of field work, it is likely that their use in the soy production in South America has increased in recent years, and will continue to increase. In South America, neonicotinoids are often applied in combination with pyrethroids for control of hemipteran pests in soy. In Argentina, there are at least 57 neonicotinoid/pyrethroid mixture formulations registered for this purpose, although not all of them are currently in commercial use (Servicio Nacional de Sanidad y Calidad Agroalimentaria, personal communication, Dec 2013). Recent studies in soy production regions of South America detected imidacloprid in 43% of surface water samples (Argentina; de Geronimo et al. 2014) and thiamethoxam in 100% of surface water samples (Brazil; Rocha et al. 2015).

Pesticide concentrations in soy production areas of South America appear to be similar to soy production areas in the United States, although other pyrethroids were detected more frequently than cypermethrin in the US. A study conducted in 2009 analyzed 14 pyrethroids in sediment samples collected from 13 streams in agricultural areas (primarily soy production) and 23 streams in urban areas throughout the US (Hladick and Kuivila 2012). Although cypermethrin was not detected in the agricultural streams, and lambda-cyhalothrin was detected at only one site, other pyrethroids (primarily bifenthrin) were detected in 10 of the 13 samples. Pyrethroid concentrations ranged from 0.3 to 180 ng/g dw, and total pyrethroid TUs for *H. azteca* ranged from 0.01 to 2.81. Another study analyzed nine pyrethroids, chlorpyrifos, and 19 organochlorine insecticides in 20 urban streams sites and 49 agricultural (primarily soy and corn) stream sites in Illinois (Ding et al. 2010). Cypermethrin was detected at only two of the agricultural sites (maximum 28 ng/g dw), but other pyrethroids (especially permethrin) were detected more often. Chlorpyrifos was detected in three samples (maximum 35 ng/g dw), while organochlorine pesticides were detected, but only at very low concentrations, and were unlikely to cause acute toxicity. In both studies, pyrethroids were detected more often in urban streams than in agricultural streams, corresponding with previous data from California (Weston and Lydy 2010).

Previous studies have detected DDT and its degradation products in Brazilian rivers and streams, but at lower concentrations and detection frequencies than those found in the present study. Use of DDT in agriculture has been prohibited in Brazil since 1985, but use for vector control was reported until 1997 (Dores 2015). In sampling conducted in rivers and streams of the northeastern Pantanal in 1999-2000, Laabs et al. (2002) found DDT and DDE in 79% and 36% of sediment samples, with maximum concentrations of 1.5 and 1.4 ng/g dw, respectively. Lower concentrations (up to 0.6 ng/kg dw) of DDT and DDE were found in a study conducted earlier in sediments of rivers in Parana state (Matsushita et al. 1996). More recent studies have detected DDT only sporadically and DDE occasionally in sediment and water of the Pantanal (Dores 2015).

Aquatic toxicity

Toxic units

Although pyrethroid concentrations were similar to other frequently detected insecticides, the TU values for these insecticides were higher because of their higher acute toxicity (Table 3). Lambda-cyhalothrin was the insecticide with the highest TU value (1.77 in Paraguay in January 2013), and TU values above 0.5 were found in four of seven sampling events in the three intensive soy production regions. Maximum cypermethrin TU values were consistently above 0.5 in the Arrecifes region during the three 2012 sampling events, as well as in the 2014 sampling event in Brazil. Bifenthrin had a maximum TU value of 0.36 (Arrecifes Feb 2014), and all other detected pyrethroids had maximum TU values less than 0.1. Endosulfan TU values were always below 0.4, but were generally higher than those of chlorpyrifos. Chlorpyrifos had the highest detection frequency in all regions and during all sampling periods, but always at low concentrations, with a maximum TU value of 0.16 (Arrecifes in March 2012). All TU values for DDT and its degradation products were less than 0.005.

In the three intensive soy production regions, pyrethroid TU values contributed more than other insecticides to the total insecticide TU values, while in the mixed use region of La Plata, endosulfan and chlorpyrifos contributed more. The maximum pyrethroid TU for all regions was 1.85 (Paraguay, January 2013), and maximum pyrethroid TU values for each sampling event exceeded 0.5 for all sampling events in the three intensive soy production regions. The maximum total insecticide TU values ranged from 0.54 to 1.89 in the intensive soy production regions, and from 0.07 to 0.66 in the mixed use La Plata region. In the intensive soy production regions, the maximum pyrethroid TU value contributed 46 to 98% of the maximum total insecticide TUs, while in the La Plata region, it contributed 7 to 71% of the total TUs.

Although maximum total TU values for each sampling event often exceeded one, the mean total TU values for each sampling event were always below 1, and for all regions except for Arrecifes they were always below 0.5. No sampling event had more than two samples with TU values that exceeded one.

Effects of synergists and insecticide mixtures

Of the insecticides found in the present study, the pyrethroids posed the highest potential for acute toxicity to aquatic invertebrates, and toxicity caused by pyrethroids may be exacerbated by the co-occurrence of PBO in streams. The LC50s used to calculate the TU values for most insecticides in the present study were based on toxicity to *H. azteca* (Table 3).Generally, *H. azteca* mortality has been found to increase when the TU of total pyrethroids reaches 0.5, and approaches 100% mortality at a TU of about 10 (Weston and Lydy 2010). Because PBO inhibits mixed-function oxidase enzymes, it acts as a synergist for pyrethroids, which are detoxified by this pathway. However, PBO can reduce toxicity of organophosphates such as chlorpyrifos, which require activation by mixed-function oxidase enzymes.PBO is often applied with pyrethrins and pyrethroids in mosquito control applications to increase their efficacy, but PBO itself has low toxicity to aquatic organisms (Amweg et al. 2006). Weston et al. (2006) found that PBO applied for mosquito control resulted in water concentrations that were high enough to increase the toxicity of pyrethroids already present in stream sediments. For example, PBO concentrations of 2-4 µg/L nearly doubled the toxicity of sediments to *H. azteca*. Amweg et al. (2006) found that a PBO sediment concentration of 12.5 ng/g and 2.3 µg/L in water almost doubled the toxicity of permethrin to *H. azteca*; however, they did not test the effect of PBO added to sediment only. The PBO concentrations detected in the present study were likely to increase the toxicity of pyrethroids in the sediment to some extent, but with existing information it was not possible to quantify the increase because of the lack of dose response data for PBO synergism with pyrethroids in sediment.

Almost all samples in the three intensive soy production regions contained multiple insecticides from at least two different insecticide classes (Tables 2 and 3), leading to uncertainty in the estimation of toxic effects. While combined effects of insecticides in the same class can be predicted relatively well, combined effects of mixtures of multiple classes are more difficult to predict (Lydy et al. 2004). At the concentrations measured in the present study, it is unlikely that either endosulfan or chlorpyrifos alone would cause significant acute toxicity to most aquatic organisms, but they could contribute to acute toxicity when occurring with other pesticides. While pesticides of similar classes and same mode of action are generally assumed to act via concentration addition, pesticides with different modes of action may act via independent action, antagonistically (less than additive toxicity), or synergistically (more than additive toxicity) (Trimble et al. 2009). In the streams examined in the present study, pyrethroids were likely to contribute more than other insecticides to acute toxicity in aquatic invertebrates, and the concentration addition model (sum of TUs) is reasonably predictive of pyrethroid mixture toxicity (Trimble et al. 2009).

There is mixed evidence on synergism and antagonism among the three classes of insecticides frequently detected together in the present study (pyrethroids, organophosphate pesticides, such as chlorpyrifos, and cyclodiene pesticides, such as endosulfan) (Ahmad 2009; Belden and Lydy 2006). Based on available data, the actual toxicity caused by multiple insecticides is not likely to exceed twice the toxicity predicted by the summed TU values (Deneer 2000).

Chronic and community level effects

Given that multiple insecticides have been consistently found in stream sediments in the present study and others in the region, it is likely that long-term chronic toxicity to aquatic organisms is occurring in the region. Both acute and chronic effects may result in changes in the invertebrate communities, notably reduction in abundances of the most sensitive taxa and increases in the most tolerant taxa. Van Wijngaarden et al. (2005) reviewed mesocosm and microcosm studies on pesticides and found that for pyrethroids, limited short-term effects tended to occur in the range of 0.01 – 0.1 TU, while clear and prolonged effects tended to occur in the range of 0.1 – 1 TU. Schäfer et al. (2012) found effects to relative abundances of sensitive macroinvertebrate taxa at pesticide concentrations lower than 1/1000 of the median effect concentration (EC50) for *Daphnia magna*. Thus, at the range of pyrethroid TU values found in soy production regions in the present study (sampling event means of 0.13 to 0.46, maximums of 0.41 to 1.85) it is likely that there would be widespread chronic and persistent effects on the aquatic invertebrate communities.

Riparian buffer widths

The highest insecticide concentrations in sediments in all intensive soy production regions occurred when buffer zone widths were 20m or less. Total insecticide TU values were compared with minimum buffer width measured immediately upstream of each site studied in the three intensive soy production regions (Figure 2). All samples with total insecticide TU values greater than 1 were collected from sites with minimum buffer widths of 20m or less.

A stepwise multiple regression for the Brazil data set indicated that buffer width was the predictor variable that had the greatest influence on total insecticide TU. Although variance inflation factors for all predictors variables were low, the correlation matrix showed percent sediment fines to be moderately correlated with three other predictors (correlation 0.45 – 0.57), and also had the highest variance inflation factor (3.6); therefore, percent sediment fines was dropped from the analysis. As a result of the AIC stepwise regression, catchment size was also eliminated as its contribution was not important in explaining variance in the TU values. The selected model included the following predictor variables: buffer width, percent total organic carbon, and stream gradient ($r^2 = 0.54$; p-value = 0.009). The analysis of relative contribution indicated that buffer width contributed 74 % of the explained variance, with percent total organic carbon and stream gradient contributing 9 and 17 %, respectively.

The results of the present study corroborate findings from other studies that have found riparian buffer zones to be important in mitigating transport of pesticides to streams. The present study's finding of the highest TU values in streams with buffer widths less than 20 m was within the range of buffer widths (5 m to 20 m) reported to mitigate pesticide effects on streams (Rasmussen et al. 2011; Di Marzio 2010; Bunzel et al. 2014; Reichenberger et al. 2007). Many factors could affect the buffer width necessary to protect streams from pesticide exposure, including gradient, type of vegetation, soil properties, types of pesticides applied, timing and amount of pesticides applied, and presence of tile drains or drainage ditches that short-circuit the buffer zones (Reichenberger et al. 2007; Bunzel et al. 2014).

Although regulation of pesticide mitigation measures often focuses on application practices, landscape level mitigation measures, such as requiring riparian buffer zones, may be easier to implement and enforce. Bereswill et al. (2014) reviewed the efficacy and practicality of risk mitigation measures for diffuse pesticide entry into aquatic ecosystems, and ranked riparian buffer strips as highly effective for mitigating both spray drift and runoff, with high acceptability and feasibility. However, the implementation and enforcement of new riparian buffer requirements in Brazil has been difficult and controversial, especially in regions with small-scale production where a significant amount of a landowner's productive farmland could be lost with compliance (Alvez et al. 2012).

Conclusions

The results of the present study demonstrated that: (1) there was consistency in the insecticides that were most commonly detected in sediment samples from streams in the intensive soy production regions studied in Argentina, Brazil and Paraguay; (2) these insecticides, especially the pyrethroids, persisted in stream sediments at concentrations likely to cause acute and chronic toxicity to aquatic invertebrates; and, (3) acutely toxic insecticide concentrations in bed sediments were most likely to occur in streams with buffer widths less than 20 m. Although frequency of detection differed somewhat between sampling events, the insecticides that were reported to be the most commonly used in soy production were also the ones that were found most frequently in all regions (e.g. chlorpyrifos, endosulfan, cypermethrin, and lambda-cyhalothrin). In addition, the pyrethroid synergist PBO was frequently detected in all three intensive soy production regions, although its use in soy production has not been reported in the literature. These results suggest that the following recommendations should be considered in soy production regions of South America: (1) evaluation and implementation of buffer zones and other management practices to limit transport of pesticides to streams; (2) field studies focusing on effects to aquatic invertebrate communities; and, (3) continued monitoring that is adapted to include the most recent pesticides being used (e.g. increasing use of neonicotinoids).

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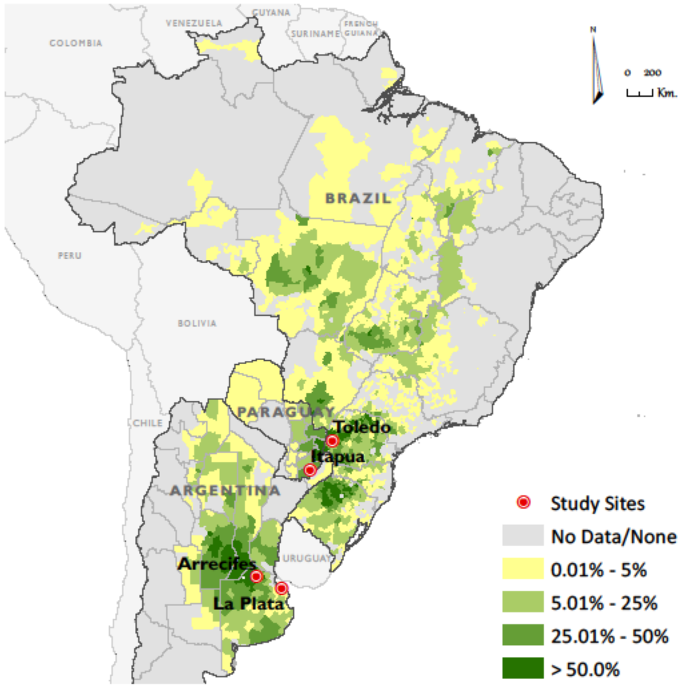

Figure 1. Study regions and soy production intensity as percent of total land use by province or department in Argentina, Brazil, and Paraguay based on data reported by governments (Argentina: http://www.minagri.gob.ar; Brazil: http://www.ibge.gov.br; Paraguay: http://www.mag.gov.py)

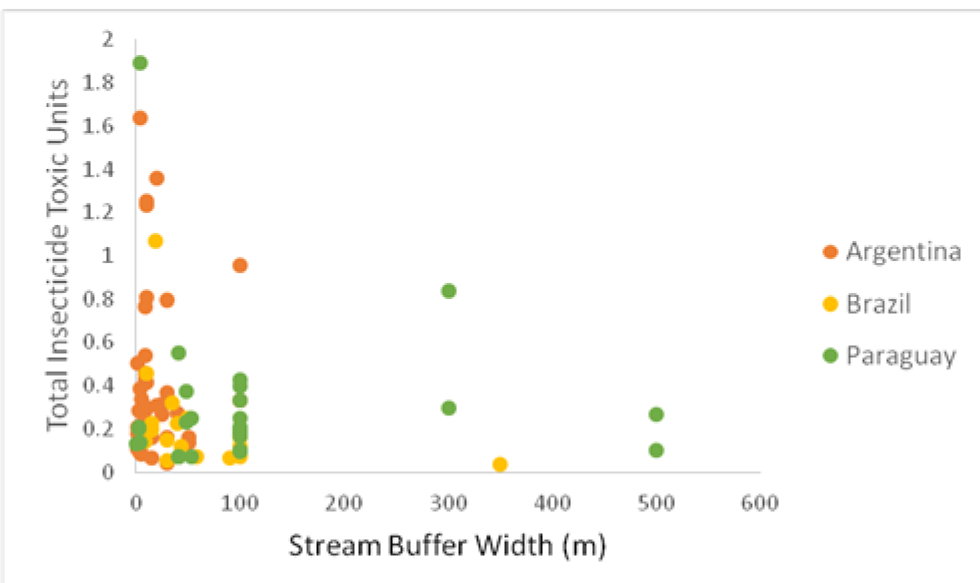

Figure 2. Relationship between riparian buffer width and total insecticide toxic units for all sites in the three intensive soy production regions studied in Brazil, Paraguay, and Argentina.

Table 1
Sediment concentrations of the most heavily used insecticides, by sampling event. Mean and standard deviation values were calculated by assigning a value of half the QL for non-detect results and for detections below the QL.

Region and Date			La Plata			Arrecifes					Paraguay		Brazil
			Dec 2011	Mar 2012	Apr 2012	Jan 2012	Mar 2012	Apr 2012	Feb 2013	Feb 2014	Jan 2013[a]	Dec 2013	Nov 2013
Method			GC-ECD	GC-ECD	GC-ECD	GC-ECD	GC-ECD	GC-ECD	GC/MS	GC/MS	GC-ECD and GC/MS	GC/MS	GC/MS
Quantitation limit (ng/g dw)			0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.25	0.5 (GC-ECD) / 0.25 (GC/MS)	0.25	0.25
Number of samples[b]			7	7[c]	4	6	7	5	12[d]	10[e]	8[f] (GC-ECD) / 8[b] (GC/MS)	14[g]	18[h]
Chlorpyrifos	k_{oc}[i] 995 - 31,000	% samples > 0.5 ng/g dw	29%	57%	100%	86%	100%	20%	100%	75%	56%	77%	83%
		Maximum (ng/g dw)	4.88	7.41	1.42	2.67	3.56	2.02	2.50	2.61	1.26	1.24	1.47
		Mean ± sd (ng/g dw)	1.21 ± 1.79	2.67 ± 2.89	0.92 ± 0.34	1.35 ± 0.83	1.94 ± 0.98	0.69 ± 0.88	1.26 ± 0.54	0.87 ± 0.71	0.50 ± 0.27	0.68 ± 0.26	0.72 ± 0.32
Endosulfan	350 - 19,953	% samples > 0.5 ng/g dw	29%	14%	0%	57%	43%	60%	8%	25%	13%	0%	0%
		Maximum (ng/g dw)	31.88	4.05	-	1.37	2.12	1.42	1.05	4.42	0.85	0.25	0.49
		Mean ± sd (ng/g dw)	7.71 ± 13.13	0.79 ± 1.44	-	0.69 ± 0.44	0.85 ± 0.84	0.66 ± 0.55	0.19 ± 0.27	0.33 ± 0.37	0.26 ± 0.19	0.13 ± 0.04	0.14 ± 0.09
Endosulfan Sulfate	320,000	% samples >0.5 ng/g dw	29%	14%	0%	29%	57%	40%	58%	33%	6%	8%	0%

		Maximum (ng/g dw)	155.50	37.64	-	4.98	6.19	1.67	12.03	2.19	0.58	0.52	0.47
		Mean ± sd (ng/g dw)	33.88 ± 61.38	5.59 ± 14.13	-	1.06 ± 1.76	1.48 ± 2.14	0.88 ± 0.74	1.53 ± 0.57	0.60 ± 0.63	0.22 ± 0.12	0.22 ± 0.15	0.21 ± 0.12
Cypermethrin	20,800 - 503,000	% samples >0.5 ng/g dw	29%	0%	0%	29%	29%	40%	33%	8%	31%	8%	44%
		Maximum (ng/g dw)	1.94	-	-	8.32	4.16	2.68	1.85	0.89	1.18	1.22	4.94
		Mean ± sd (ng/g dw)	0.67 ± 0.72	-	-	1.61 ± 3.01	1.23 ± 1.70	0.86 ± 1.22	0.64 ± 0.64	0.21 ± 0.23	0.45 ± 0.32	0.24 ± 0.22	0.88 ± 1.20
Lambda Cyhalothrin	>80,000 - 182,000	% samples >0.5 ng/g dw		0%	0%		29%	40%	17%	0%	6%	8%	39%
		Maximum (ng/g dw)		-	-		6.09	5.05	0.63	0.42	16.57	1.22	1.32
		Mean ± sd (ng/g dw)		-	-		1.12 ± 2.19	1.45 ± 2.40	0.28 ± 0.20	0.23 ± 0.11	1.22 ± 4.10	0.13 ± 0.26	0.50 ± 0.30

[a] Two different analytical methods were used for this sampling event, and statistics are based on all 16 sample
[b] Statistics include all samples including those with low surrogate recovery, low MS/MSD recovery, or high RPD.
[c] 2 samples had surrogate recovery <50%
[d] MS sample had <50% recovery for endosulfan
[e] MS/MSD samples had <50% recovery and/or RPD was > 25% for endosulfan and chlorpyrifos
[f] 4 samples had surrogate recovery < 50%, and MS/MSD samples had <50% recovery for endosulfan and chlorpyrifos
[g] 10 samples had surrogate recovery < 50%, and MS/MSD samples had <50% recovery for endosulfan and chlorpyrifos
[h] RPD was > 25% for chlorpyrifos and cypermethrin
[i] Range of koc values reported at http://toxnet.nlm.nih.gov/

Table 2. Maximum sediment concentrations and detection frequencies of additional compounds analyzed in 2013 and 2014 (GC/MS, quantitation limit 0.25 ng/g dw)

		Arrecifes		Paraguay		Brazil
		Feb 2013	Feb 2014	Jan 2013	Dec 2013	Nov 2013
Number of samples	K_{oc}[a]	12[b]	10[c]	8[b]	14[d]	18[e]
PBO	399 – 830	3.31 (92%)	2.91 (33%)	1.87 (88%)	1.23 (8%)	11.14 (94%)
Bifenthrin	131,000 - 302,000	nd	2.96 (17%)	0.37 (38%)	0.63 (31%)	1.44 (44%)
Permethrin	10,471 - 86,000	0.47 (8%)	0.47 (15%)	2.56 (13%)	nd	2.07 (33%)
Cyfluthrin	3,700 to 33,913	<0.25 (8%)	nd	<0.25 (13%)	0.40 (38%)	<0.25 (11%)
DDD	130,600 - 131,800	nd	7.26 (25%)	nd	nd	3.97 (33%)
DDE	26,300 - 75,860	nd	nd	nd	1.88 (15%)	5.67 (100%)
DDT	113,000 - 350,000	nd	0.29 (8%)	nd	0.49 (23%)	1.06 (100%)
Esfenvalerate	5,248	<0.25 (8%)	nd	<0.25 (38%)	nd	0.29 (22%)
Endrin Ketone	11,420	nd	nd	<0.25 (13%)	nd	0.34 (6%)
Alpha Chlordane	20,000 - 76,000	nd	0.33 (8%)	nd	<0.25 (8%)	nd
Deltamethrin	79,000 - 16,300,000	<0.25 (8%)	nd	<0.25 (13%)	nd	0.87 (6%)
Aldrin	400 - 28,000	nd	nd	nd	nd	0.42 (11%)
Heptachlor Epoxide	7800	nd	<0.25 (8%)	nd	nd	nd
Gamma Chlordane	20,000 - 76,000	nd	0.32 (8%)	nd	nd	nd
Endrin	11,420	nd	nd	nd	<0.25 (8%)	nd

[a] Range of koc values reported at http://toxnet.nlm.nih.gov/
[b] MS/MSD samples had <50% recovery and/or RPD was > 25% for the following pesticides: lindane, endrin, dieldrin, heptachlor, aldrin, chlordane, DDD, DDE, DDT.
[c] MS/MSD samples had <50% recovery and/or RPD was > 25% for the following pesticides: lindane, endrin, heptachlor, aldrin, chlordane, tefluthrin, DDD, DDE, and DDT.
[d] 10 samples had surrogate recovery < 50%, and MS/MSD samples had <50% recovery and/or RPD was > 25% for the following pesticides: lindane, heptachlor, aldrin, chlordane, tefluthrin, and deltamethrin.
[e] RPD was > 25% for cyfluthrin and deltamethrin

Table 3. Maximum and mean toxic units (TUs) for each sampling event, for pesticides that had at least one TU value >0.01.TUs were calculated as the ratio of the carbon-normalized concentration in sediment over the carbon-normalized LC50.

			La Plata (Argentina)			Arrecifes (Argentina)					Paraguay		Brazil
Pesticide	**LC50 (ng/g organic carbon)**	**Statistic**	**Dec 2011**	**Mar 2012**	**Apr 2012**	**Jan 2012**	**Mar 2012**	**Apr 2012**	**Feb 2013**	**Feb 2014**	**Jan 2013**	**Dec 2013**	**Nov 2013**
Chlorpyrifos	4160[a]	Maximum	0.01	0.02	0.01	0.06	0.16	0.04	0.09	0.08	0.15	0.05	0.02
		Mean	0.00	0.01	0.01	0.03	0.06	0.02	0.03	0.03	0.04	0.02	0.01
Endosulfan	960[b]	Maximum	0.32	0.04	nd	0.14	0.18	0.37	0.01	0.09	0.01	0.04	0.02
		Mean	0.08	0.01	nd	0.07	0.08	0.11	0.00	0.03	0.00	0.02	0.00
Endosulfan Sulfate	5220[b]	Maximum	0.28	0.07	nd	0.08	0.10	0.08	0.12	0.03	0.05	0.01	0.01
		Mean	0.06	0.01	nd	0.02	0.03	0.02	0.02	0.01	0.03	0.01	0.00
Cypermethrin	380[a]	Maximum	0.05	nd	nd	1.15	0.97	0.58	0.38	0.13	0.19	0.27	0.83
		Mean	0.02	nd	nd	0.28	0.27	0.20	0.16	0.03	0.06	0.10	0.11
Lambda-cyhalothrin	450[a]	Maximum		0.02	nd		0.71	0.93	0.23	0.16	1.77	0.61	0.16
		Mean		0.01	nd		0.17	0.26	0.04	0.07	0.12	0.11	0.05
Bifenthrin	520a	Maximum							nd	0.36	0.00	0.14	0.13
		Mean							nd	0.04	0.00	0.05	0.03
Permethrin	10830[a]	Maximum							0.00	0.00	0.02	0.01	0.01
		Mean							0.00	0.00	0.00	0.00	0.00
Cyfluthrin	1080[a]	Maximum							<QL	nd	<QL	0.05	<QL
		Mean							<QL	nd	<QL	0.02	<QL
Deltamethrin	790[a]	Maximum							nd	0.00	<QL	nd	0.06
		Mean								0.00	<QL	nd	0.00
Esfenvalerate	1540[a]	Maximum							<QL	nd	<QL	nd	0.01
		Mean							<QL	nd	<QL	nd	0.00
Total pyrethroid TU [c, e]		Maximum	0.05	0.05	0.05	1.15	1.16	1.51	0.45	0.41	1.85	0.77	1.03

	Mean	0.02	0.02	0.03	0.28	0.44	0.46		0.13	0.19	0.28	0.20
Total insecticide TU [d, e]	Maximum	0.66	0.14	0.07	1.23	1.36	1.64	0.96	0.54	1.89	0.84	1.07
	Mean	0.16	0.05	0.05	0.40	0.61	0.60		0.20	0.26	0.34	0.21

[a] LC50 for *Hyalella azteca* from Weston et al. 2013
[b] LC50 for *Chironomus tentans* from You et al. 2005
[c] Total pyrethroid TU values for each sample were calculated by summing the TU values for each pyrethroid.
[d] Total insecticide TU values for each sample were calculated by summing the TU values for each insecticide.
[e] A concentration value of half the QL was assigned for pesticides not detected, or detected <QL.

Table S1

Sampling Sites and Schedule

Site Name	Country	Region	Habitat Type	Regional Land Use	Latitude	Longitude	Year 1				Year 2		Year 3		
							Dec-11	Jan-12	Mar-12	Apr-12	Jan-13	Feb-13	Nov-13	Dec-13	Feb-14
Remes	Argentina	La Plata	Pampas	Mixed agriculture/ grazing	35 1 31.87S	57 59 39.6W	X		X						
Poblet	Argentina	La Plata	Pampas	Mixed agriculture/ grazing	35 2 2.45S	57 56 34.3W	X		X						
Pescado	Argentina	La Plata	Pampas	Mixed agriculture/ grazing	35 1 23.97S	57 51 27.42W	X		X						
Cajaravilla	Argentina	La Plata	Pampas	Mixed agriculture/ grazing	35 4 6.37S	57 48 57.17W	X		X	X					
Blanco	Argentina	La Plata	Pampas	Mixed agriculture/ grazing	35 8 30.23S	57 26 23.98W	X		X	X					
Destino	Argentina	La Plata	Pampas	Mixed agriculture/ grazing	35 8 15.35S	57 23 41.21W	X		X	X					
Arregui	Argentina	La Plata	Pampas	Mixed agriculture/ grazing	35 7 38.83S	57 41 39.01W	X		X	X					
H0	Argentina	Arrecifes	Pampas	Intensive soy production	34 8 31.58S	59 50 31.74W		X	X	X					
H1	Argentina	Arrecifes	Pampas	Intensive soy production	34 10 6.13S	59 49 57.32W		X							
H2	Argentina	Arrecifes	Pampas	Intensive soy production	34 10 19.46S	59 50 42.60W		X	X						
H5	Argentina	Arrecifes	Pampas	Intensive soy production	34 734.67S	59 50 14.31W						X			X
A3	Argentina	Arrecifes	Pampas	Intensive soy production	34 10 56.82S	59 58 56.13W			X	X					
A1	Argentina	Arrecifes	Pampas	Intensive soy production	34 7 28.59S	60 3 30.76W		X	X	X					
A2	Argentina	Arrecifes	Pampas	Intensive soy production	34 10 42.52S	59 59 23.43W			X	X					X
Tres Horquetas	Argentina	Arrecifes	Pampas	Intensive soy production	34 2 52.40S	59 56 40.00W						X			X
Canete	Argentina	Arrecifes	Pampas	Intensive soy production	34 1 53.64S	60 8 5.50W		X	X			X			X
Contador 2	Argentina	Arrecifes	Pampas	Intensive soy production	34 9 20.13S	60 4 51.35W						X			X
Gomez	Argentina	Arrecifes	Pampas	Intensive soy production	34 7 38.72S	59 54 1.50W		X				X			X
H. Bonar	Argentina	Arrecifes	Pampas	Intensive soy production	34 18 14.30S	60 20 0.78W						X			X
Helves 2	Argentina	Arrecifes	Pampas	Intensive soy production	34 2 53.30S	60 0 56.71W						X			X

Las Animas	Argentina	Arrecifes	Pampas	Intensive soy production	34 6 59.39S	60 12 32.90W					X			X
Los Ingleses	Argentina	Arrecifes	Pampas	Intensive soy production	33 59 10.67S	60 11 59.21W					X			X
Luna 2	Argentina	Arrecifes	Pampas	Intensive soy production	34 11 54.76S	60 1 31.78W								X
Maguire	Argentina	Arrecifes	Pampas	Intensive soy production	33 55 19.70S	60 16 5.90W	X	X	X		X			X
Salto 2	Argentina	Arrecifes	Pampas	Intensive soy production	34 11 11.90S	60 14 7.22W					X			X
BR-02	Brazil	Toledo	Atlantic forest	Intensive soy production	24 48 44.4S	53 42 31.6W						X		
BR-03	Brazil	Toledo	Atlantic forest	Intensive soy production	24 48 51.4S	53 38 22.2W						X		
BR-07	Brazil	Toledo	Atlantic forest	Intensive soy production	24 57 31.2S	53 40 53.5W						X		
BR-10	Brazil	Toledo	Atlantic forest	Intensive soy production	24 56 54.4S	53 41 52.5W						X		
BR-11	Brazil	Toledo	Atlantic forest	Intensive soy production	24 56 31.8S	53 42 49.1W						X		
BR-12	Brazil	Toledo	Atlantic forest	Intensive soy production	24 45 49.1S	53 48 55.7W						X		
BR-13	Brazil	Toledo	Atlantic forest	Intensive soy production	24 44 41.6S	53 51 40.3W						X		
BR-14	Brazil	Toledo	Atlantic forest	Intensive soy production	24 44 40.8S	53 51 59.3W						X		
BR-15	Brazil	Toledo	Atlantic forest	Intensive soy production	24 45 28.2S	53 52 55.8W						X		
BR-16	Brazil	Toledo	Atlantic forest	Intensive soy production	24 45 05.2S	53 53 03.6W						X		
BR-17	Brazil	Toledo	Atlantic forest	Intensive soy production	24 44 57.4S	53 54 18.2W						X		
BR-18	Brazil	Toledo	Atlantic forest	Intensive soy production	24 47 43.0S	53 54 11.0W						X		
BR-19	Brazil	Toledo	Atlantic forest	Intensive soy production	24 49 19.8S	53 54 12.2W						X		
BR-20	Brazil	Toledo	Atlantic forest	Intensive soy production	24 49 07.5S	53 50 29.2W						X		
BR-21	Brazil	Toledo	Atlantic forest	Intensive soy production	24 48 29.6S	53 45 44.8W						X		
BR-22	Brazil	Toledo	Atlantic forest	Intensive soy production	24 45 48.2S	53 37 54.1W						X		
BR-23	Brazil	Toledo	Atlantic forest	Intensive soy production	24 47 48.0S	53 36 16.8W						X		
BR-24	Brazil	Toledo	Atlantic forest	Intensive soy production	24 47 57.2S	53 36 01.7W						X		
SD 01	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 42 21.2S	55 31 49.1 W				X			X	
SD 02	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 45 48.7S	55 33 34.7W				X				

SD 03	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 49 34.5S	55 31 36.8W	X	
SD 04	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 53 29.8S	55 34 19.1W	X	X
SD 05	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 55 54.1S	55 31 00.8W	X	X
SD 06	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 52 12.5S	55 29 49.7W	X	X
SD 07	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 37 45.8S	55 39 55.1W	X	X
SD 08	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 47 37.0S	55 37 33.4W	X	X
SD 09	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 47 59.4S	55 35 38.0W	X	X
SD 10	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 50 08.3S	55 36 33.2W	X	X
SD 11	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 50 35.0S	55 36 43.7W	X	
SD 12	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 31 29.1S	55 37 06.7W	X	
SD 13	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 32 18.4S	55 37 0.8W	X	X
SD 14	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 26 19.0S	55 34 32.4W	X	X
SD 15	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 30 56.5S	55 36 43.3W		X
SD 16	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 28 21.9S	55 34 07.9W	X	X
SD 17	Paraguay	Itapua	Atlantic forest	Intensive soy production	26 28 27.90S	55 33 57.40W		X

Table S2

Summary statistics of site characteristics in each region

		Argentine Pampas		Converted Atlantic forest	
		La Plata [a]	Arrecifes	Paraguay	Brazil
Maximum depth (m)	minimum	0.20	0.25	0.20	0.12
	maximum	>2	0.80	1.20	0.80
	median	0.50	0.60	0.41	0.25
	mean	0.62	0.56	0.48	0.33
	standard deviation	0.26	0.12	0.23	0.19
Maximum width (m)	minimum	0.6	3.0	2.5	1.0
	maximum	25.0	8.0	12.0	10.0
	median	10.0	5.0	5.0	4.0
	mean	11.8	5.7	5.7	4.0
	standard deviation	7.6	1.5	2.1	1.8
Maximum velocity (m/s)	minimum		0.06	0.18	0.24
	maximum		0.85	1.20	1.98
	median		0.34	0.40	0.58
	mean		0.36	0.49	0.63
	standard deviation		0.20	0.27	0.36
Estimated flow (m3/s)	minimum		0.04	0.01	0.01
	maximum		0.69	1.05	0.41
	median		0.15	0.15	0.06
	mean		0.21	0.20	0.11
	standard deviation		0.17	0.20	0.11
Gradient (%)	minimum		0.1	0.7	0.8
	maximum		0.5	8.7	11.1
	median		0.3	2.0	3.1
	mean		0.3	2.6	4.5
	standard deviation		0.1	2.1	3.5
Catchment size (Ha)	minimum		1824	224	75
	maximum		28659	3591	5042
	median		4124	1115	731
	mean		6790	1537	919
	standard deviation		7258	1196	1102
% Cultivated (footnote b)	minimum			31.60	74.35
	maximum			82.30	93.37
	median			73.60	89.38
	mean			70.96	87.66
	standard deviation			11.55	5.27
Minimum buffer distance (m)	minimum		1.0	3.0	9.0
	maximum		100.0	500.0	350.0

	median		10.0	100.0	32.5
	mean		19.7	89.3	56.6
	standard deviation		23.0	93.4	87.3
Water conductivity (uS/cm)	minimum	220	663	24	14
	maximum	4000	1796	163	61
	median	921	895	61	28
	mean	906	961	66	31
	standard deviation	813	247	24	13
Water temperature (C)	minimum		18.3	17.6	19.0
	maximum		30.5	24.0	22.8
	median		22.9	21.0	20.7
	mean		23.1	20.7	20.6
	standard deviation		3.0	1.4	1.0
Water pH	minimum	6.7	7.0	5.9	4.5
	maximum	9.0	9.1	7.4	7.6
	median	7.4	8.0	6.8	6.7
	mean	7.7	7.9	6.8	6.6
	standard deviation	0.7	0.5	0.3	0.7
Dissolved oxygen (mg/L)	minimum	4.00	4.90	2.90	7.30
	maximum	12.90	18.77	10.00	14.60
	median	9.30	10.27	8.42	8.50
	mean	8.48	10.78	8.37	8.79
	standard deviation	2.6	3.2	1.0	1.3
Water turbidity	minimum		3.9	5.5	3.2
	maximum		96.0	37.5	31.0
	median		14.7	11.2	16.1
	mean		21.9	13.9	16.8
	standard deviation		22.2	6.4	7.1
% sediment TOC	minimum	2%	0.26	0.22	1.37
	maximum	12%	2.00	2.12	3.24
	median	10%	1.16	0.55	2.39
	mean	8%	1.16	0.69	2.32
	standard deviation	4%	0.52	0.44	0.68
% sediment fines (silt and clay)	minimum		52.7	10.6	44.6
	maximum		78.1	86.3	83.1
	median		64.7	38.6	65.7
	mean		65.5	42.0	65.9
	standard deviation		8.6	18.7	11.6

[a] La Plata statistics don't include temperature data because it was only collected for some samples. Other parameters are blank because measurements were not taken

[b] Paraguay/Brazil - cultivated area is based on non-forested area, estimated with LANDSAT data

Table S3

Analytical parameters

Compound	Molecular weight	Retention time (min)	Target ion (*m/z*)	Qualifier ions (*m/z*)	Qualifier ions (*m/z*)
Alpha Lindane	288	10.061	255	257	253
Beta Lindane	288	10.406	255	257	253
Gamma Lindane	288	10.489	255	257	253
Tefluthrin	418	10.741	241	243	205
Delta Lindane	288	10.835	255	257	253
Heptachlor	370	10.985	266	232	237
Aldrin	362	12.102	237	330	239
Chlorpyrifos	349	12.097	313	214	315
Heptachlor Epoxide	386	12.714	318	237	388
Gamma Chlordane	406	13.09	266	304	NA
Endosulfan I	404	13.308	372	374	NA
Alpha Chlordane	406	13.333	266	304	NA
DDE	316	13.615	318	316	320
Dieldrin	378	13.726	380	346	237
Endrin	378	14.009	380	346	308
Endosulfan II	404	14.251	406	408	NA
DDD	318	14.309	248	355	357
DDT	352	14.936	71	73	75
Endosulfan Sulfate	420	14.936	386	422	NA
Endrin Ketone	378	15.757	308	345	382
Bifenthrin	422	15.769	386	381	206
Lambda Cyhalothrin	449	16.696	241	205	243
Permethrin Cis	390	17.415	207	209	354
Permethrin Trans	390	17.971	207	209	354
Cyfluthrin 1	433	17.964	207	171	209
Cyfluthrin 2	433	18.051	207	171	209
Cyfluthrin 3&4	433	18.135	207	171	209
Cypermethrin 1	415	18.283	207	171	209
Cypermethrin 2	415	18.372	207	171	209
Cypermethrin 3&4	415	18.463	207	171	209
Esfenvalerate	419	19.578	211	213	212
Deltamethrin	503	20.304	297	299	295

CHAPTER 3

Acute toxicity of four insecticides to the South American amphipod *Hyalella curvispina* based on sediment and water exposures

Acute toxicity of four insecticides to the South American amphipod *Hyalella curvispina* based on sediment and water exposures

Abstract

Cypermethrin, lambda-cyhalothrin, chlorpyrifos, and endosulfan are insecticides with very high aquatic toxicity that are commonly used in soy production and other large scale crop systems in South America. For each of these insecticides, in both sediment and water, we determined median lethal concentration (LC50) values for *Hyalella curvispina*, a freshwater amphipod that is widespread in South America and is closely related to *H. azteca,* a standard test species in the United States. We then calculated species sensitivity distributions (SSDs) for freshwater invertebrate taxa using results of this study and other available data. Results of sediment and water toxicity tests were consistent, with the lowest L50 values for lambda-cyhalothrin, followed by cypermethrin, chlorpyrifos and alpha-endosulfan. Although the younger age class of neonates (1-2 wks) was more sensitive to both pyrethroid insecticides than the older age class (2-3 wks), the younger age class was less practical for bioassays because of high variability in control survival and difficulty finding live organisms when terminating sediment tests. The SSD resultsfor the four pesticides tested indicated that the sensitivity of *H. curvispina* is similar to that of *H. azteca,* and that both organisms are more sensitive than most other freshwater invertebrate taxa.

Introduction

Cypermethrin, lambda-cyhalothrin, chlorpyrifos and endosulfan are insecticides with very high aquatic toxicity that are commonly used in soy production and other large scale crop systems in South America(Hunt et al., 2016). In recent years, soybean production has become a major export crop for multiple countries in South America, including Brazil, Argentina, Paraguay, Uruguay, and Bolivia (Castanheira and Freire, 2013; Garrett et al., 2013). Lepidoptera pests are often controlled by applications of chorpyrifos, an organophosphate, and Hemiptera pests by endosulfan, an organochlorine. Pyrethroids, especially cypermethrin and lambda-cyhalothrin, are commonly used for both types of pests, and are often applied at the same time as other pesticides (Di Marzio et al., 2010; Organismo Provincial para el Desarrollo Sostenible, 2013). Multiple studies have found high detection rates of these pesticides in both sediment and water in South American streams, at concentrations that are toxic to aquatic organisms (Di Marzio et al., 2010; Hunt et al., 2016; Jergentz et al., 2004b; Marino and Ronco, 2005; Mugni et al., 2011; Possavatz et al., 2014).

As insecticides are increasingly used in South America and other parts of the southern hemisphere, the question has arisen as to whether water quality standards based on toxicity data for northern hemisphere taxa are adequately protective. The vast majority of aquatic toxicity testing for pesticides is performed on species from Europe and North America, and toxicity data for species native to the southern hemisphere are lacking. For example, Hagen and Douglas (2014) compiled laboratory toxicity data to compare sensitivity of Australian marine invertebrates to northern hemisphere species, and found sufficient data to compare only three out of 109 chemical substances.

Hyalella curvispina is a freshwater amphipod that is widespread in South America, and it has been utilized in toxicity testing for over a decade, but more data are needed to establish pesticide toxicity threshold values for this species. Another species of the same genus, *H. azteca*, is native to North America and is a standard test species in the United States (United States Environmental Protection Agency, 2000). *H. azteca* is known to be very sensitive to many pesticides including pyrethroids and chlorpyrifos, therefore it is often used in aquatic toxicity testing when these pesticides are expected to be present in water bodies (Weston et al., 2009). *Hyalella* species are especially useful in toxicity testing because they can be used in both sediment and water toxicity tests (United States Environmental Protection Agency, 2000; Weston and Lydy, 2010).

The insecticides most commonly used in soy production in South America (chlorpyrifos, endosulfan, cypermethrin, and lambda-cyhalothrin) have low water solubility, and a high affinity to bind to soil and sediments based on chemical properties (Hunt et al., 2016). Sediment LC50 values for *H. azteca* have been published for all four of these insecticides (Weston et al., 2013; You et al., 2004), and freshwater LC50 values for *H. azteca* have been published for three of them (Ding et al., 2012; Wan et al., 2005; Donald P. Weston et al., 2013). However, no sediment LC50 values have been published for *H. curvispina*, although previous studies have published LC50 values for some insecticides in water(Mugni et al., 2013, 2012).

To utilize *H. curvispina* as a sensitive surrogate species in pesticide toxicity testing, it is important to determine the most sensitive life stage that can be used in bioassays. *H. azteca* toxicity tests are generally conducted with neonates that are between 7-14 days old when tests are started because this life stage has been demonstrated to be more sensitive than older ones in testing of a limited number of toxicants (United States Environmental Protection Agency, 2000). However, many published studies on *H. curvispina* toxicity testing have used juveniles or adults (Mugni et al., 2013, 2012, 2011).

The objectives of this study were to: (1) establish LC50 values for *H. curvispina* for the chlorpyrifos, alpha-endosulfan, cypermethrin, and lambda-cyaholothrin in both sediment and water; (2) compare the sensitivity of *H. curvispina* to *H. azteca* and other freshwater aquatic invertebrates; and (3) compare the sensitivity of different ages groups of *H. curvispina*, and evaluate their practicality for use in bioassays.

Materials and Methods

Test materials

H. curvispina was collected from the El Destino stream in a relatively undeveloped area located in a reserve 25 km south of the city of La Plata, Argentina. Organisms were acclimated for at least one week prior to use in bioassays. They were cultured in plastic containers filled with reconstituted water prepared according to recommendations for *H. azteca* (United States Environmental Protection Agency, 2000). Lettuce leaves were placed in culture containers to serve as substrate and food, supplemented by formulated fish food every two days.

Sediment was collected from the same location as *H. curvispina* collection, and thoroughly homogenized prior to taking an aliquot for analysis of insecticides. None of the insecticides analyzed (chlopyrifos, endosulfan, cypermethrin and lambda-cyhalothrin) were detected in sediment samples. Organic carbon content was 1.7%, and moisture content measured just prior to spiking was 3.6%.

Sediment and water toxicity bioassays

The 96 h water and 10 d sediment acute toxicity bioassays generally followed the methods of Weston et al. (2009) and Weston and Jackson (2009), except for substituting formulated fish food for yeast/cerophyll/trout chow for the specified feeding regimes (1 feeding at 48 h for water tests, daily feeding for sediment tests). A 16 h:8 h light:dark photocycle was used, and temperature was maintained at 23 °C. Five to seven concentration steps were used for each toxicant, with each step varying by a factor of 2. Spiked sediment samples were prepared approximately 1 week prior to starting bioassays, and stored in a refrigerator after thorough homogenization by hand. Spiked water was prepared immediately prior to beginning bioassays. Each concentration was prepared by adding the appropriate amount of toxicant dissolved in acetone, with < 32 µL/L acetone in water and < 1.1 µg/g acetone in sediment. Solvent controls were spiked with the maximum amount of acetone that was used in the concentrations series. For each pesticide concentration and the control, tests were conducting using three replicate glass beakers, each containing 10 *H. curvispina* individuals.

For water bioassays, 80 mL of spiked reconstituted water and a substrate consisting of a 1 cm^2 nylon screen was included in each beaker. After 48 h exposure, 1 mL of formulated fish food was added to each beaker, and a 6 h feeding period was provided. Then ~80% of the water was removed from the beaker and replaced with fresh sample. After an additional 48 h (96 h total), the test was terminated and survivors counted.

To compare sensitivity of different ages, separate water bioassays were conducted for two groups: (1) organisms that passed through a 500 µm sieve but did not pass through a 355 µm sieve (i.e. those approximately 1-2 weeks old); and (2) organisms that passed through a 710 µm sieve but did not pass through a 500 µm sieve (i.e. approximately 2-3 weeks old). An additional size class (organisms that did not pass through a 710 µm sieve, and were greater than 3 weeks old) was tested for lambda-cyhalothrin. Organisms were sieved 3 – 5 d prior to initiating bioassays.

For sediment bioassays, 70g of spiked sediment and 200 mL of reconstituted water were added to each beaker. We selected test organisms that passed through a 710 µm sieve but did not pass through a 355 µm sieve (i.e. approximately 1-3 weeks old). Organisms were sieved 5-6 d prior to initiating bioassays. Twice a day 80% of the water was removed from the beaker and replaced with fresh reconstituted water. 1 mL of formulated fish food was added to each beaker daily just after the first water change.

Mortality data were used to estimate the LC50 values and 95% confidence intervals by probit analysis using the USEPA Benchmark Dose Software (USEPA 2015).

Species Sensitivity Distributions

To compare the sensitivity of *H. curvispina*to sensitivities of *H. azteca* and other aquatic invertebrate taxa, we calculated species sensitivity distributions (SSDs) for each insecticide, using the United States Environmental Protection Agency (USEPA) SSD Generator software (United States Environmental Protection Agency, 2015a). SSDs use available toxicity data for multiple species tested with a given chemical to derive a joint sensitivity distribution, from which the proportion of species affected by a certain concentrations can be estimated by the quantile of the distribution (Smetanová et al., 2014). The USEPA SSD Generator software produces SSDs by fitting the log-probit distribution to toxicity data (United States Environmental Protection Agency, 2015a)

To calculate SSDs for each insecticide examined in this study, we used LC50 values obtained for *H. curvispina* from the present study, as well as all acute (1 to 4d) water toxicity data for freshwater invertebrates available in the USEPA ECOTOX database, including mortality and immobilization endpoints (United States Environmental Protection Agency, 2015b). Because cypermethrin results for *H. azteca* were not included in the ECOTOX database, we supplemented the cypermethrin SSD data set with a 4d immobilization EC50 value (Weston and Jackson, 2009).

Only one study in the ECOTOX database reported LC50 values for *H. curvispina*(Mugni et al., 2012). Instead of combining data from that study with the present study, we used separate data points for each the two studies in the SSD. We kept them separate so that we could compare the relative sensitivities of *H. curvispina* obtained from our study results with those of Mugni et al. 2012. For all other taxa, results from all studies of the relevant insecticide were combined into a single data point by using the mean of all reported LC50 values for each taxa for input into the SSD (United States Environmental Protection Agency, 2015a).

Adequate water toxicity data were available in the ECOTOX database for three of the four insecticides: 146 taxa had data available for chlorpyrifos, 71 taxa for endosulfan, and 56 taxa for cypermethrin. For lambda-cyhalothrin, LC50 data were available for only three taxa in addition to *H. curvispina*. Therefore, we also calculated a SSD for the insecticide gamma-cyhalothrin, for which data were available for13 taxa. SSDs were generated only for water toxicity, because insufficient data were available to calculate SSDs for sediment toxicity.

Results

Acute toxicity to Hyalella curvispina

Results of sediment and water toxicity tests were consistent, with the lowest LC50 values for lambda-cyhalothrin, followed by cypermethrin, chlorpyrifos, and alpha-endosulfan (Table 1). Water temperatures measured in all control replicates twice a day ranged between 22.4– 23.9 °C for water bioassays, and 21.4 – 24.5 °C for sediment bioassays.

The LC50 values for both cypermethrin and lambda-cyahothrin were lower for the smaller size class, and confidence intervals did not overlap (Table 1). When the largest age class (2-3 weeks old) was tested with lambda-cyhalothrin in water using the same concentration series as the other two age classes, there was no significant mortality even at the highest concentration (12 ng/L). Survival in control replicates for water bioassays using organisms sieved with sizes 500-710 μm ranged from 90-100%, with a mean of 97 – 100% for each test. For water bioassays using smaller organisms (sieve size 355-500um), control mortality was higher, with survival rates ranging from 30-100% for individual replicates and 70-100% for means of all control replicates. For endosulfan, LC50 values could not be calculated for the younger age class because of high variability in both controls and dilution series.

Species Sensitivity Distributions

The SSD for chlorpyrifos (Figure 1a) indicates that *H. curvispina* is more sensitive than 94% of species, based on the *H. curvispina* LC50 value determined in the present study, and more sensitive than 96% of species based on the *H. curvispina* LC50 value determined previously (Mugni et al., 2012). More sensitive organisms tested include *H. azteca*, the mayflies *Deleatidium sp.* and *Procloeon sp*.,the cladocerans *Daphnia ambigua* and *Ceriodaphnia dubia*, and the dragonfly *Pseudagrion spp*. *D. ambigua* is known to be native to South America, and *H. azteca* occurs in just the northernmost part of South America.

The SSD for endosulfan (Figure 1b) indicates that *H. curvispina* is more sensitive than 56% of species, based on the *H. curvispina* LC50 value determined in the present study, and more sensitive than 78% of species based on the *H. curvispina* LC50 value determined previously (Mugni et al., 2012). However, the present study used only the alpha endosulfan isomer, which is more toxic than the typical endosulfan isomer mixture that was used in the and likely in most other reported results. *H. azteca* is more sensitive than 67% of species, based on results from only one study. Of the 15 species tested that were more sensitive than *H. curvispina* based on results of the present study, all were crustaceans except for two mayflies (*Atalophlebia australis* and *Jappa kutera*) and one caddisfly (*Cheumatopsyche sp.*).

The SSD for cypermethrin (Figure 1c) indicates that *H. curvispina* is more sensitive than 87% of species, based on the *H. curvispina*LC50 value determined in the present study, and more sensitive than 89% of speciesbased on the *H. curvispina* LC50 value determined previously (Mugni et al., 2012). The4d immobilization EC50 value reported by Weston and Jackson (2009) indicates that *H. azteca* is more sensitive than 99% of all species, and was the most sensitive of all species tested. Taxa that were more sensitive than *H. curvispina* include the mayfly *Baetis rhodani*, the decapods *Macrobrachium rosenbergii, Trichodactylus borellianus*, and *Palaemonetes argentines*, and the chironomid *Tanytarsus sp*. All of these species belong to genera that are common in South America.

For lambda-cyhalothrin, LC50 data were available for only three other taxa, and *H. curvispina* was more sensitive than all of them (Figure 1d), based on results of the present study. No other studies included LC50 values for *H. curvispina* or *H. azteca*. The SSD estimates that *H. curvispina* is more sensitive than 87% of all species. There is high uncertainty in this estimate given the limited amount of data, but it corresponds well with the SSD generated for gamma-cyhalothrin (Figure 1e), which indicates that *H. azteca* is more sensitive than 88% of species (there were no data available for *H. curvispina* for this compound).

Discussion

The results of this study indicate that for the four pesticides tested, the sensitivity of *H. curvispina* is similar to that of *H. azteca*, and that both organisms are more sensitive than most other freshwater invertebrate taxa. Results of this study are also in close agreement with previous data the sensitivity of *H. curvispina*, although there were some differences in the methods. Although for *H. azteca* bioassays, neonates of 7-14d are recommended for use (United States Environmental Protection Agency, 2000), we found that it was impractical to use organisms of *H. curvispina*at this young age because of high mortality in controls.

We hypothesized that LC50 values generated in the present study would be lower than the LC50 values for *H. curvispina* previously reported by Mugni et al. (2012) because we used younger organisms and ran the bioassays for a longer duration. Mugni et al. (2012) used larger organisms (5-10 mm length), which would be expected to be less sensitive than the younger organisms used in the present study (approximately 1-2 mm length). In addition, the shorter duration bioassays (2d) conducted by Mugni et al. (2012) would be expected to result in higher LC50 values than those conducted over 4d in the present study. Because the test populations for the two studies were both collected from the same undeveloped stream in Argentina, it is not expected that there would be a difference in sensitivity between the two laboratory populations.

Contrary to expectations, the LC50 values calculated by Mugni et al. (2012) for synthetic water were lower for chlorpyrifos (60 ng/L) and cypermethrin (10 ng/L), and confidence intervals did not overlap with those of the present study. The LC50 values for endosulfan were not directly comparable between the two studies because different isomers were used, and Mugni et al. (2012) did not evaluate lambda-cyhalothrin.

There were two main differences in methods used in the Mugni et al. (2012) study and the present study that may have contributed to lower sensitivity in the present study. First, Mugni et al. (2012) maintained the water temperature at 18 °C, while the present study maintained temperature at 23 °C. Lower temperatures are known to increase toxicity of pyrethroids, but decrease toxicity of organophosphates (Weston and Lydy, 2010), so temperature could explain the difference for cypermethrin but not for chlorpyrifos. Mugni et al. (2013) repeated the experiments for 48d cypermethrin LC50 determinations three times, this time at a higher temperature (22 ±2 °C), and this time found LC values (33-96 ng/L, mean of 65 ng/L) that are higher than that reported in the present study (34.5 ng/L). Second, the present study included a mesh substrate material in each replicate, which allows the organisms to rest by clinging to the substrate. Mugni et al. (2012) and Mugni et al. (2013) used no substrate, which may have increased the organisms' sensitivity by depriving them of rest.

Despite the differences in the LC50 values from *H. curvispina*studies, the results indicate very similar relative placements of *H. curvispina* in the SSDs, and conclude that this species is generally very sensitive to insecticides compared to most other taxa. For chlorpyrifos and cypermethrin, the SSD placements for LC50 values calculated in the present study and by Mugni et al. (2012) differby only two percentile points.

The relative sensitivities of *H. azteca* and *H. curvispina* in water testswere also very similar, with *H. azteca* usually determined to be more sensitive. However, recent studies (Clark et al., 2015; Weston et al., 2013) indicate that field populations of *H. azteca* tend to be less sensitive than laboratory cultures, with a difference of about two orders of magnitude. The differences in sensitivity have been hypothesized to result from genetic adaptation, nongenetic changes in enzyme activity, or both (Clark et al., 2015; D. P. Weston et al., 2013).

For sediment tests, no LC50 values have previously been reported for *H. curvispina*, and few other species have been tested. However, sediment LC50 values have been reported for *H. azteca* for all four insecticides, and results correspond well with LC50 values for *H. curvispina* from the present study. The 10-d sediment LC50 values for *H. curvispina*determined in the present study for chlorpyrifos, alpha-endosulfan, and lambda-cyhalothrin were slightly lower that reported for *H. azteca* (4160 ng/g OC for chlorpyrifos(Weston et al., 2013); 51,700 ng/g OC for alpha-endosulfan (You et al., 2004); and 450 ng/g OC for lambda-cyhalothrin(Weston et al., 2013)).

The 10-d sediment LC50 for *H. curvispina*determined for cypermethrin in the present study was 2073 ng/g OC, higher than that reported for *H. azteca* (380 ng/g OC (Weston et al. 2013)).

Although the younger age group of H. curvispina was somewhat more sensitive to the pyrethroid insecticides, organisms of this size were problematic to use in bioassays because of variable survival in controls. In addition to the variability in controls, we found that it was impractical to use the younger age category for sediment bioassays because it required many hours to search through the sediment for the small organisms when terminating bioassays.

This study adds information on pesticide sensitivity of an important South American amphipod species, and indicates that its sensitivity is very similar to a closely related North America species. A repeated criticism by the scientific community in South Americais the lack of sensitivity data on species that occur outside of Europe and North America, and the application of sensitivity data for northern hemisphere species to South American taxa. There may be reason to believe that sensitivities could be different in species occurring in the Southern hemisphere and near the equator. For example, Kwok et al. (2007) found that tropical species may be more sensitive than temperate species to pesticides, while temperate species are likely to be more sensitive to metals. More studies are needed on a range of organisms to determine whether use of sensitivity data for northern hemisphere species are adequately protective for southern hemisphere species.

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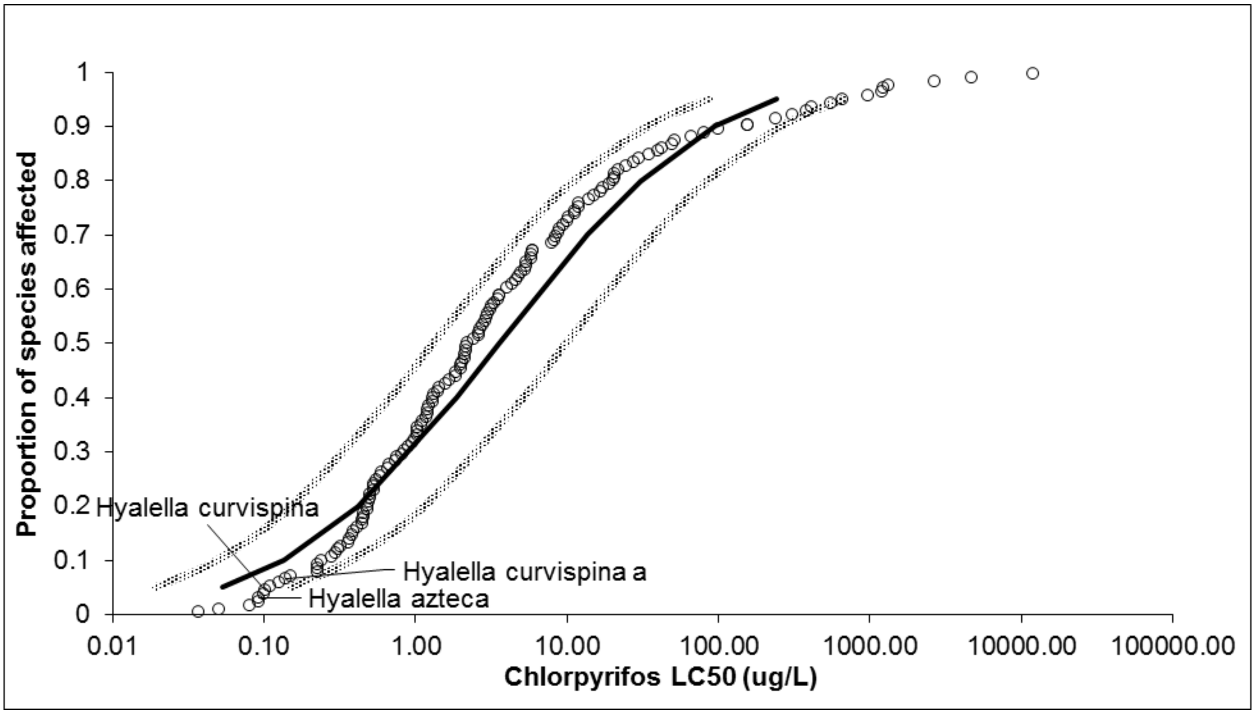
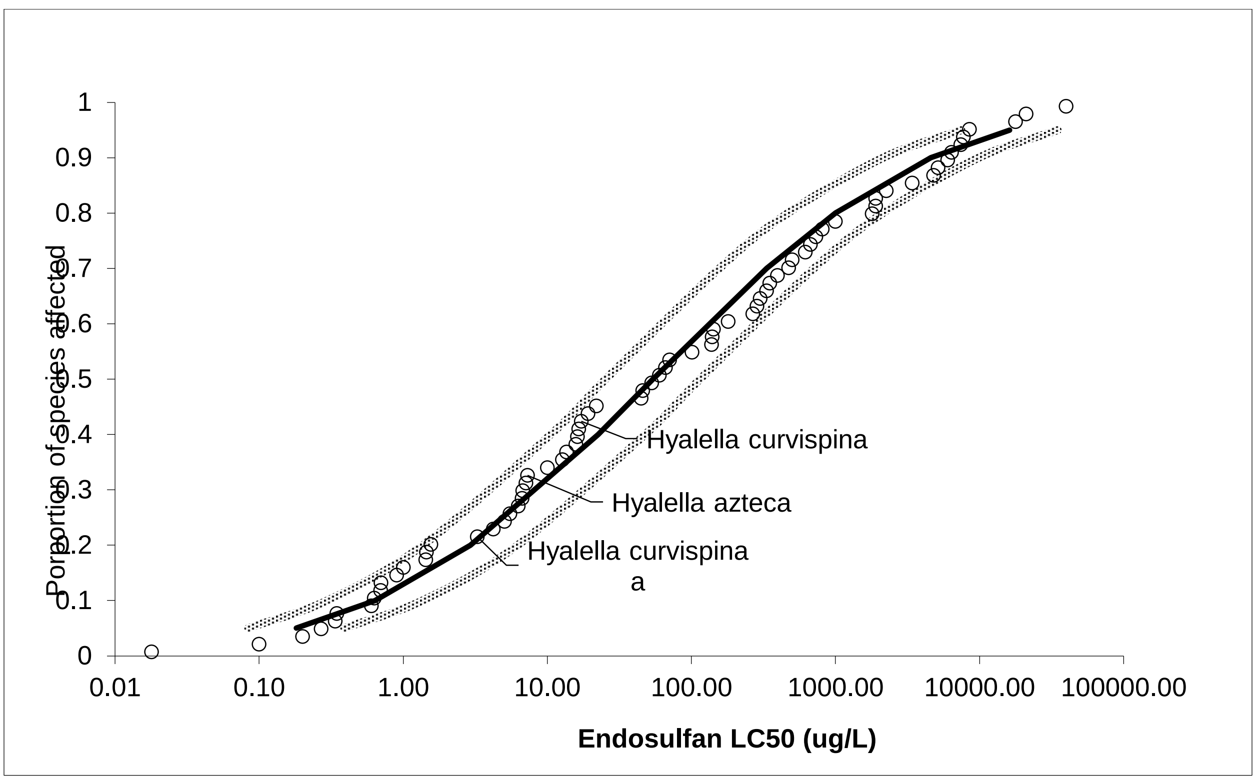
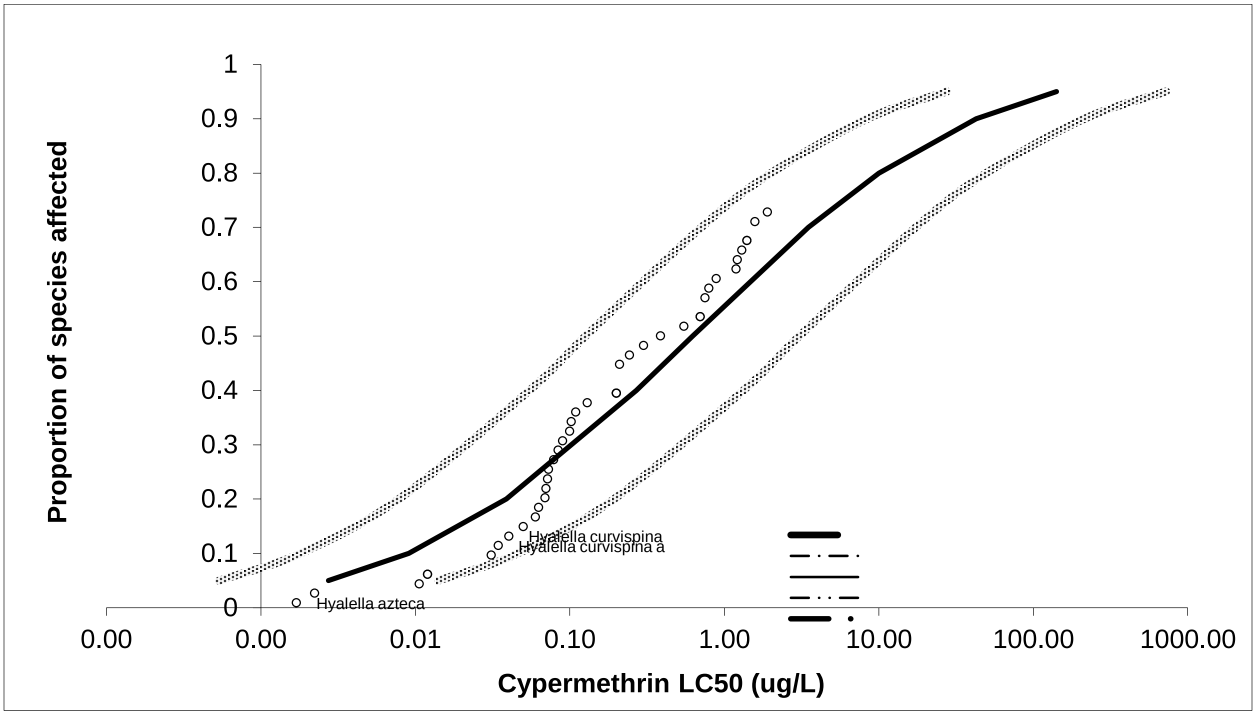
(a)


Proportion of species affected
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Hyalella curvispina
Hyalella curvispina a
Hyalella azteca
0.01
0.10
1.00
10.00
100.00
1000.00
10000.00
100000.00
Chlorpyrifos LC50 (ug/L)

(b)

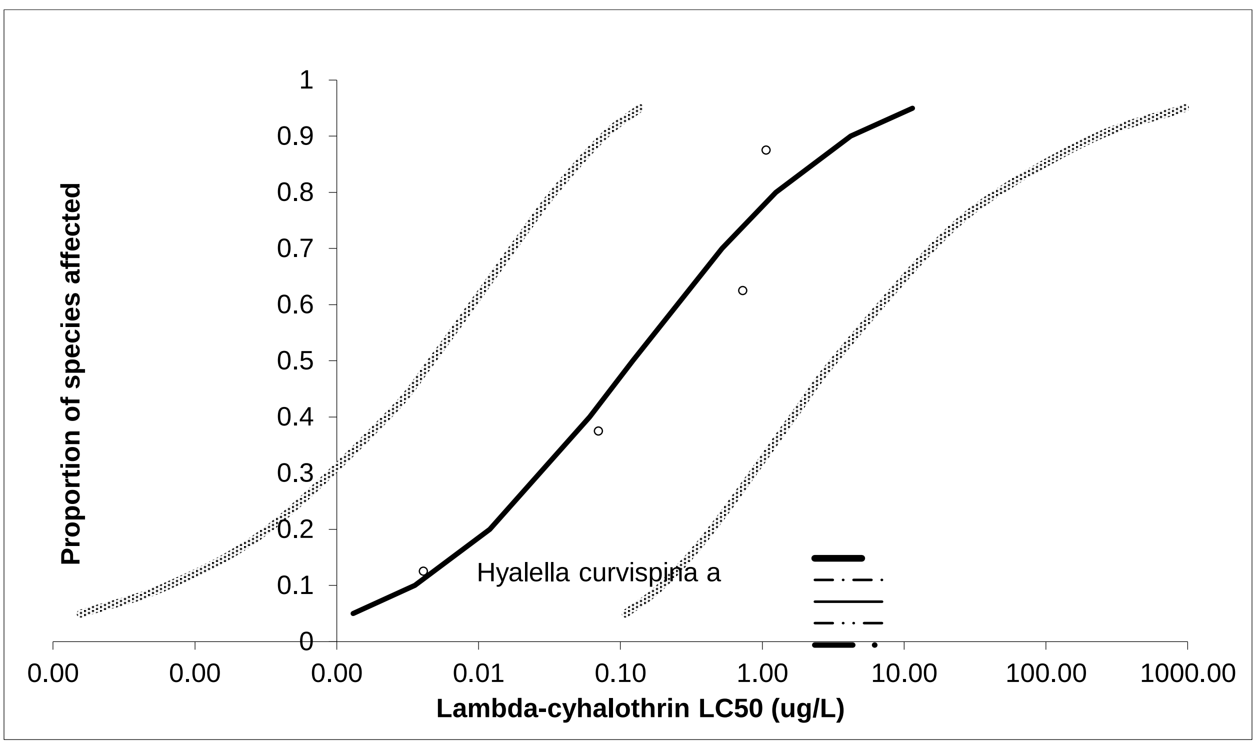

Proportion of species affected
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Hyalella curvispina
Hyalella azteca
Hyalella curvispina
a
0.01
0.10
1.00
10.00
100.00
1000.00
10000.00
100000.00
Endosulfan LC50 (ug/L)

(c)



Proportion of species affected
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Hyalella curvispina
Hyalella curvispina a
Hyalella azteca
0.00
0.00
0.01
0.10
1.00
10.00
100.00
1000.00
Cypermethrin LC50 (ug/L)

(d)



Proportion of species affected
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Hyalella curvispina a
0.00
0.00
0.00
0.01
0.10
1.00
10.00
100.00
1000.00
Lambda-cyhalothrin LC50 (ug/L)

(e)

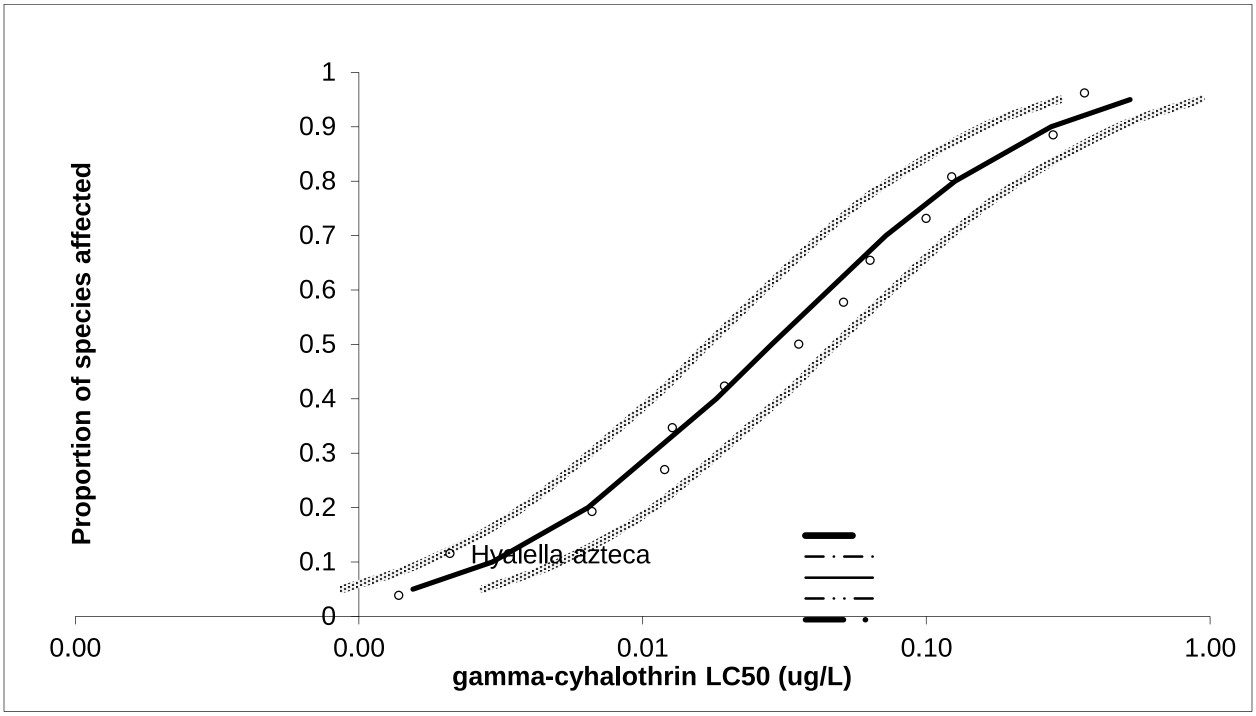


Figure 1 - Species sensitivity distributions for (a) chlorpyrifos, (b) endosulfan, (c) cypermethrin, (d) lambda-cyhalothrin, and (e) gamma-cyhalothrin. Points represent all LC50 values for freshwater invertebrates from the USEPA ECOTOX database (mean values for each taxon). The solid black line represents the central tendency value, and gray lines indicate the upper and lower prediction intervals (United States Environmental Protection Agency, 2015a). See Appendix A for LC50 values and relative sensitivity values for each taxon.

Table 1. *Hyalella curvispina* LC50 values (with 95% confidence intervals) for four insecticides for 96 hr water bioassays (two age groups), and for 10d sediment bioassays.

	Water LC50s (ng/L)		**Sediment LC50s (ng/g)**
	sieve size 355-500 µm (1-2 weeks old)	sieve size 500-710 µm (2-3 weeks old)	Sieve size 355-710 µm (1-3 weeks old)
L-cyhalothrin	4.1 (3.2-5.1)	13.5 (9.8-26.5)	256 (197-334)
Cypermethrin	34.5 (25.7-42.1)	71.8 (61.6-83.8)	2,073 (1,644-2,758)
Chlorpyrifos	138 (119-160)	142 (112-170)	3,101 (2,327-3,674)
Endosulfan I		3,264 (2,784-3831)	33,342 (25,185-40,424)

Appendix A. Species Sensitivity Distribution Results

Table A1.Species Sensitivity Distribution Results for Chlorpyrifos

Taxa	LC50 (µg/L)	Standard Deviation	Proportion of Taxa More Sensitive	Number of Observations
Daphnia ambigua	0.036946	0.0332	0%	2
Deleatidium sp.	0.05		1%	1
Procloeon sp.	0.081		2%	1
Ceriodaphnia dubia	0.091412	0.2222	2%	34
Hyalella azteca	0.092198	0.2910	3%	19
Pseudagrion sp.	0.1		4%	1
Hyalella curvispina [a]	0.100995	0.3198	4%	2
Amphipoda	0.11		5%	1
Moina australiensis	0.126491	0.1444	6%	2
Hyalella curvispina [b]	0.138		6%	1
Palaemonetes pugio	0.15	0.0000	7%	2
Daphnia carinata	0.224094	0.2478	8%	7
Gammarus pseudolimnaeus	0.224309	0.1457	9%	3
Paratya australiensis	0.226429	0.4059	9%	14
Culex sitiens	0.24		10%	1
Atalophlebia australis	0.277936	0.0886	11%	4
Aedes taeniorhynchus	0.298896	0.4630	11%	3
Simocephalus vetulus	0.308616	0.3536	12%	6
Gammarus lacustris	0.322173	0.4275	13%	3
Ephemerella sp.	0.363318	0.0591	13%	2
Daphnia pulex	0.364845	0.5216	14%	13
Daphnia longispina	0.383366	0.2130	15%	4
Culex restuans	0.392251	0.0805	15%	3
Gammarus pulex	0.415126	0.5834	16%	12
Chironomus dilutus	0.444773	0.2196	17%	5
Culex tritaeniorhynchus ssp. summorosus	0.449024	0.2891	17%	6
Simulium vittatum	0.454623	0.8908	18%	7
Macrobrachium rosenbergii	0.458258	0.2602	19%	2
Aedes togoi	0.48		19%	1
Cloeon dipterum	0.483631	0.2381	20%	14
Palaemonetes argentinus	0.49		21%	1
Aedes sticticus	0.5		21%	1
Chironomus sp.	0.501996	0.1096	22%	2
Culex tarsalis	0.529704	0.3225	23%	5
Thamnocephalus platyurus	0.53		23%	1
Aedes triseriatus	0.531553	0.3751	24%	4
Aedes canadensis	0.548635	0.2761	25%	2
Caenis horaria	0.591608	0.1034	26%	2

Aedes atropalpus	0.6		26%	1
Aedes vexans	0.66494	0.1596	27%	3
Culex pipiens ssp. pipiens	0.665843	0.3303	28%	3
Culex pipiens ssp. molestus	0.736022	0.3654	28%	5
Chaoborus obscuripes	0.758045	0.4308	29%	12
Leptoceridae	0.818474	0.0375	30%	2
Peltodytes sp.	0.848528	0.0362	30%	2
Paramelita nigroculus	0.9		31%	1
Macrobrachium lar	0.960937	0.3540	32%	2
Anopheles quadrimaculatus	1		32%	1
Culex peus	1.036008	0.0836	33%	4
Culex pipiens ssp. quinquefasciata	1.036072	0.2487	34%	13
Culex melanurus	1.036976	0.3525	34%	4
Aedes cantans	1.1		35%	1
Anopheles stephensi	1.122868	0.4023	36%	8
Anisops sardeus	1.192476	0.1728	36%	2
Aedes communis	1.2		37%	1
Daphnia magna	1.215647	1.0583	38%	32
Globodera rostochiensis	1.220312	0.1109	38%	4
Chaoborus americanus	1.29		39%	1
Chironomus tepperi	1.3		40%	1
Gammarus fasciatus	1.338656	0.8789	40%	2
Maxillopoda	1.414991	0.2512	41%	2
Pteronarcella badia	1.421571	0.5292	42%	3
Anopheles freeborni	1.578642	0.2199	43%	12
Tanypus nubifer	1.688194	0.7474	43%	2
Chironomus tentans	1.834675	1.3076	44%	8
Molanna angustata	1.86	0.0000	45%	2
Chironomus utahensis	1.981156	0.3771	45%	3
Procambarus acutus ssp. acutus	2		46%	1
Claassenia sabulosa	2.033857	0.5808	47%	3
Laccophilus fasciatus	2.1	0.0000	47%	2
Romanomermis culicivorax	2.1		48%	1
Sigara arguta	2.16		49%	1
Heptageniidae	2.165153	1.4505	49%	3
Neoplea striola	2.203838	0.2399	50%	7
Caenis sp.	2.45		51%	1
Procladius sp.	2.608552	1.2426	51%	3
Corixa punctata	2.633397	0.2253	52%	5
Aedes punctor	2.7		53%	1
Macrobrachium lanchesteri	2.791385	0.0569	53%	4
Anax imperator	2.872559	0.2289	54%	8
Culiseta incidens	2.924396	0.1439	55%	6
Culicoides variipennis	3.023348	0.1277	55%	11

Anopheles gambiae	3.108054	0.4899	56%	2
Plea minutissima	3.193917	0.3218	57%	8
Aedes excrucians	3.3		57%	1
Culiseta annulata	3.5		58%	1
Limnephilus indivisus	3.6		59%	1
Diaptomus forbesi	3.6	0.0000	59%	2
Triops longicaudatus	4		60%	1
Chironomus riparius	4.394836	2.4422	61%	3
Laccophilus maculosus ssp. maculosus	4.6		62%	1
Culex tritaeniorhynchus	4.752715	0.9509	62%	8
Asellus aquaticus	5.017871	0.1767	63%	9
Enallagma sp.	5.28		64%	1
Streptocephalus sudanicus	5.358171	0.2650	64%	2
Chaoborus punctipennis	5.4	0.0000	65%	2
Crocothemis erythraea	5.8		66%	1
Procambarus sp.	5.800453	0.6560	66%	16
Thermonectus basillaris	6		67%	1
Orconectes immunis	6		67%	1
Anopheles albimanus	8		68%	1
Ranatra linearis	8.237088	0.3241	69%	8
Xanthocnemis zealandica	8.44		70%	1
Culex pipiens	8.724777	1.1026	70%	51
Berosus styliferus	9		71%	1
Notonecta maculata	9.420363	0.3148	72%	7
Plecoptera	10		72%	1
Sialis lutaria	10.19641	1.8716	73%	5
Parapoynx stratiotata	11.30302	0.5326	74%	8
Odonata	11.4		74%	1
Glyptotendipes paripes	12		75%	1
Culex pipiens ssp. pallens	12.06382	1.4928	76%	13
Culex quinquefasciatus	13.99863	1.3164	77%	45
Belostoma sp.	15		77%	1
Dicrotendipes californicus	16.7332	0.5353	78%	2
Aedes aegypti	17.32702	0.8703	79%	41
Goeldichironomus holoprasinus	19.19896	1.8335	79%	2
Tropisternus lateralis	20.39608	0.5749	80%	2
Tanytarsus sp.	20.78461	2.0653	81%	2
Pteronarcys californica	20.80084	0.3537	81%	3
Chironomus decorus	21.94948	1.0747	82%	6
Hydrophilus triangularis	24.4949	0.1245	83%	2
Procambarus clarkii	27.63574	0.1376	83%	5
Hydropsychidae	30		84%	1
Notonecta undulata	35.2	0.0000	85%	2
Hygrotus sp.	40		85%	1

Cricotopus sp.	42.36752	0.7095	86%	3
Chironomus plumosus	49.62969	0.6733	87%	15
Chironomus crassicaudatus	52		87%	1
Branchiura sowerbyi	66	0.0000	88%	2
Eriocheir sinensis	80.23455	0.4301	89%	8
Hydrophilus sp.	100		89%	1
Spiralothelphusa hydrodroma	156.8908	0.0879	90%	4
Parathelphusidae	156.8908	0.0879	90%	4
Trichodactylus borellianus	242.32		91%	1
Dugesia japonica	307.4801	0.2316	92%	4
Lampsilis siliquoidea	381.9605	0.1623	93%	4
Neocaridina denticulata	413.9556	0.2520	94%	8
Oziotelphusa senex ssp. senex	551.9525	0.2304	94%	15
Aedes albopictus	666.3784	0.9284	95%	26
Pomacea canaliculata	978		96%	1
Agamermis unka	1210		96%	1
Zilchiopsis collastinensis	1242.54		97%	1
Bulinus truncatus	1320		98%	1
Lanistes carinatus	2710		98%	1
Anopheles sinensis	4700		99%	1
Brachionus calyciflorus	11949.83	0.0025	100%	5

[a] Results from the present study.

[b] Results reported by Mugni et al. 2012.

Table A2.Species Sensitivity Distribution Results for Endosulfan

Taxa	LC50 (μg/L)	Standard Deviation	Proportion of Taxa More Sensitive	Number of Observations
Mesocyclops longisetus	0.017889	0.0686	1%	2
Eucyclops sp.	0.1		2%	1
Alonella sp.	0.2		3%	1
Paratelphusa jacquemontii	0.269189	0.2086	5%	4
Palaemonetes paludosus	0.337263	0.1232	6%	4
Daphnia longispina	0.34641	0.0884	8%	2
Diaptomus sp.	0.6		9%	1
Atalophlebia australis	0.62967	0.0786	10%	10
Cheumatopsyche sp.	0.69755	0.2589	12%	6
Paratya australiensis	0.699714	0.1943	13%	2
Cypria sp.	0.9		15%	1
Amphipoda	1		16%	1
Jappa kutera	1.429578	0.1879	17%	16
Cypris subglobosa	1.449138	0.2278	19%	2
Caridina laevis	1.558076	0.2602	20%	2
Hyalella curvispina [a]	3.2641		22%	1
Macrobrachium lamarrei	4.211157	0.0772	23%	4
Macrobrachium dayanum	5.042816	0.0754	24%	4
Macrobrachium rosenbergii	5.491317	0.8502	26%	8
Palaemonetes argentinus	6.28		27%	1
Gammarus lacustris	6.671231	0.0952	28%	4
Pteronarcys californica	6.761489	0.5142	30%	3
Gammarus fasciatus	7.113787	0.1281	31%	3
Hyalella azteca	7.285761	0.1718	33%	3
Asellus aquaticus	10		34%	1
Caridina weberi	12.7393	0.3529	35%	16
Atalophlebia sp.	13.59562	0.0482	37%	6
Eretes sticticus	15.81139	0.2814	38%	2
Moina micrura	16.2		40%	1
Sigara alternata	16.48171	0.1997	41%	3
Hyalella curvispina[b]	17.2		42%	1
Paramelita nigroculus	19.2		44%	1
Enallagma sp.	21.87933	0.1070	45%	3
Spicodiaptomus chelospinus	44.72136	0.0686	47%	2
Pseudagrion sp.	46		48%	1
Chironomus plumosus	53		49%	1
Radix luteola	60		51%	1
Culex pipiens ssp. quinquefasciata	66		52%	1
Culex quinquefasciatus	70.70557	2.8051	53%	4
Procambarus clarkii	100.7571	0.8812	55%	2

Ischnura sp.	138.0812	0.2059	56%	8
Lamellidens marginalis	139.1637	1.7052	58%	4
Culex fatigans	142.3025	0.0324	59%	2
Lamellidens corrianus	179.8902	1.5373	60%	4
Daphnia magna	266.7614	0.4159	62%	40
Ceriodaphnia dubia	285.5834	0.6444	63%	3
Daphnia pulex	300		65%	1
Moinodaphnia macleayi	332.7537	0.2683	66%	2
Daphnia carinata	350.3941	0.2507	67%	3
Crocothemis erythraea	395		69%	1
Anopheles stephensi	473.4		70%	1
Cambarus sp.	500		72%	1
Potamonautes sp.	616.7274	0.3181	73%	4
Hydra viridissima	670		74%	1
Moina macrocopa	731.0267	0.9331	76%	2
Hydra vulgaris	810		77%	1
Aedes aegypti	1000		78%	1
Bellamya dissimilis	1800		80%	1
Zilchiopsis collastinensis	1902		81%	1
Trichodactylus borellianus	1905.96		83%	1
Barytelphusa cunicularis	2256.426	0.0078	84%	3
Lanistes carinatus	3400		85%	1
Radix natalensis	4794.393	0.0570	87%	2
Brachionus calyciflorus	5150	0.0000	88%	4
Tubifex tubifex	6000		90%	1
Physella acuta	6400		91%	1
Semisulcospira libertina	7400		92%	1
Oziotelphusa senex ssp. senex	7734.817	0.1295	94%	18
Cipangopaludina malleata	8500		95%	1
Barytelphusa guerini	17780		97%	1
Indoplanorbis exustus	21000		98%	1
Melanopsis dufouri	39891.81	0.0295	99%	3

[a] Results from the present study.

[b] Results reported by Mugni et al. 2012.

Table A3.Species Sensitivity Distribution Results for Cypermethrin

Taxa	LC50 (µg/L)	Standard Deviation	Proportion Taxa More Sensitive	Number of Observations
Hyalella azteca[a]	0.0017		1%	1
Palaemonetes argentinus	0.002236	0.0686	3%	2
Trichodactylus borellianus	0.010628	0.0451	4%	3
Tanytarsus sp.	0.012		6%	1
Baetis rhodani	0.012		6%	1
Macrobrachium rosenbergii	0.031		10%	1
Hyalella curvispina [b]	0.0345		11%	1
Hyalella curvispina[c]	0.040331	0.4467	13%	5
Piona carnea	0.05		15%	1
Tanypus nubifer	0.06		17%	1
Culex fuscocephala	0.063		18%	1
Orconectes nais	0.069		20%	1
Gyrinus natator	0.07		22%	1
Aedes vexans	0.072		24%	1
Culex restuans	0.073		25%	1
Gammarus pulex	0.078802	0.1767	27%	7
Triops longicaudatus	0.084		29%	1
Paratya australiensis	0.09		31%	1
Culex pipiens	0.099875	0.3445	32%	2
Culex sp.	0.102396	0.5050	34%	2
Cloeon dipterum	0.109545	1.0445	36%	2
Procladius sp.	0.13		38%	1
Chironomus riparius	0.2		39%	1
Chaoborus sp.	0.2		39%	1
Caecidotea brevicauda	0.2		39%	1
Chironomus utahensis	0.21		45%	1
Aedes stimulans	0.243697	0.2031	46%	3
Notonecta sp.	0.3		48%	1
Cricotopus sp.	0.387814	0.1181	50%	2
Goeldichironomus holoprasinus	0.547723	0.0560	52%	2
Glyptotendipes paripes	0.7	0.0000	54%	2
Corixa punctata	0.7		54%	1
Acartia tonsa	0.75		57%	1
Culex quinquefasciatus	0.79526	1.0619	59%	13
Ceriodaphnia dubia	0.889		61%	1
Macrobrachium lar	1.192938	0.0668	62%	2
Culex tritaeniorhynchus	1.217217	0.5894	64%	3
Heptageniidae	1.3		66%	1
Odonata	1.4		68%	1
Hydropsychidae	1.4		68%	1

Chironomus decorus	1.580316	0.2775	71%	4
Anopheles stephensi	1.9		73%	1
Culex pipiens ssp. quinquefasciata	2.02682	0.5787	75%	2
Chironomus crassicaudatus	2.244994	0.3847	76%	2
Aedes aegypti	2.561153	0.9471	78%	29
Dicrotendipes californicus	3.954744	0.0928	80%	2
Lymnaea peregra	5		82%	1
Culex pipiens ssp. pallens	5.018249	0.4680	83%	13
Daphnia magna	5.755189	1.3718	85%	19
Hydrophilus sp.	8.3		87%	1
Simulium vittatum	9.8		89%	1
Thermocyclops oblongatus	29		90%	1
Aedes albopictus	465.6174	0.6625	92%	17
Lymnaea acuminata	514.6248	0.1528	94%	4
Anopheles sinensis	1600		96%	1
Oziotelphusa senex ssp. senex	2000		97%	1
Melanoides tuberculata	5846.618	0.2663	99%	4

[a] Results reported by Weston and Jackson 2009, not included in ECOTOX database.

[b] Results from the present study.

[c] Results reported by Mugni et al. 2012.

Table A4.Species Sensitivity Distribution Results for Lambda-Cyhalothrin

Taxa	LC50 (µg/L)	Standard Deviation	Proportion of Taxa More Sensitive	Number of Observations
Hyalella curvispina a	0.0041		13%	1
Culex pipiens ssp. pallens	0.07		38%	1
Culex quinquefasciatus	0.73		63%	1
Daphnia magna	1.064315485	0.7979	88%	7

Table A5.Species Sensitivity Distribution Results for Gamma-Cyhalothrin

Taxa	LC50 (µg/L)	Standard Deviation	Proportion of Taxa More Sensitive	Number of Observations
Gammarus pseudolimnaeus	0.001382	0.4618	4%	3
Hyalella azteca	0.002094	0.4188	12%	12
Chaoborus obscuripes	0.006628	0.2104	19%	4
Gammarus pulex	0.011962	0.2966	27%	6
Notonecta maculata	0.012731	0.4573	35%	5
Corixa punctata	0.019429	0.3081	42%	5
Proasellus coxalis	0.03556	0.5364	50%	5
Cloeon dipterum	0.051165	0.7320	58%	5
Asellus aquaticus	0.063343	0.5934	65%	6
Daphnia magna	0.1		73%	1
Chironomidae	0.122826	0.1708	81%	3
Macrobrachium nipponense	0.28		88%	1
Coenagrionidae	0.3611	0.3201	96%	4

CHAPTER 4

Effects of insecticides on stream invertebrate communities in soy production regions of the Argentine Pampas

Effects of insecticides on stream invertebrate communities in soy production regions of the Argentine Pampas

Abstract

We investigated relationships among insecticides and aquatic invertebrate communities in 22 streams of two soy production regions of the Argentine Pampas over three growing seasons. Chlorpyrifos, endosulfan, cypermethrin and lambda-cyhalothrin were the insecticides most frequently detected in stream sediments. The Species at Risk (SPEAR) pesticide bioassessment index ($SPEAR_{pesticides}$)was applied to evaluate relationships between sediment insecticide toxic units (TUs) and invertebrate communities associated with both benthic habitats and emergent vegetation. The SPEAR trait thresholds for classification of taxa with respect to generation time and pesticide sensitivity were optimized for the Argentina data sets. $SPEAR_{pesticides}$ was the only response metric that was significantly correlated with total insecticide TU values for all three averaged data sets, consistently showing a trend of decreasing values with increasing TU values (r^2 = 0.35 to 0.42, p-value = 0.001 to 0.03). Although pyrethroids were the insecticides that contributed the higest TU values, toxicity calculated based on all insecticides was better at predicting changes in invertebrate communities than toxicity of pyrethroids alone. Crustaceans, particularly the amphipod *Hyalella spp.*, which are relatively sensitive to pesticides, played a large role in the performance of $SPEAR_{pesticides}$, and the relative abundance of all crustaceans also showed a significant decreasing trend with increasing insecticide TUs for two of three data sets (r2 = 0.30 to 0.57, p-value = 0.003 to 0.04). For all data sets, total insecticide TU was the most important variable in explaining variance in the $SPEAR_{pesticide}$ index. This study was the first application of the SPEAR index in South America, and the first oneto use it to evaluate effects of pesticides on invertebrate communities associated with aquatic vegetation. Although the SPEAR index was developed in Europe, it performed well in the Argentine Pampas with only minor modifications, andwould likely improve in performance as more data are obtained on South American taxa traits, such as pesticide sensitivity and generation time.

Introduction

The Argentine Pampas is the central plain of Argentina with a mild climate and very fertile soil. Previously covered by grasslands, it is now the most productive agricultural region of the country. Over the last three decades, soybeans have become a major export crop for Argentina, and increased pesticide use has led to concerns about environmental effects. For example, between 1995 and 2011, soy cultivation area expanded by 209% in Argentina (Castanheira and Freire, 2013). Most global soy production occurs in North and South America, although a large part of the soy is exported to Europe and China, primarily for use as animal feed (Garrett et al., 2013). Pesticide consumption in Argentina increased from 6 million kilograms in 1992 (Pengue, 2000) to 32 million kilograms in 2012 (CASAFE, 2013). Insecticide application rates are approximately double those of fungicides, and the insecticides most frequently used in soy production (pyrethroids, chlorpyrifos, and endosulfan) have very high aquatic toxicity (Hunt et al., 2016; Nordborg et al., 2014).

Multiple studies have detected soy production insecticides in both sediment and water collected from streams in Argentina (Di Marzio et al., 2010; Hunt et al., 2016; Jergentz et al., 2005; Marino and Ronco, 2005; Mugni et al., 2011), but there is a lack of field studies investigating effects to aquatic invertebrate communities. Several studies in Argentina have found associations between stream insecticide concentrations and effects on the native amphipod *Hyalella curvispina* using single-species toxicity tests (Di Marzio et al., 2010; Jergentz et al., 2004a; Mugni et al., 2011). Also, Jergentz et al. (2004b) found that a pulse of endosulfan was associated with reductions in abundances of Odonata and Ephemeroptera in two small Pampas streams). However, to date no study in the region has documented widespread insecticide effects on aquatic invertebrate communities.

The Species at Risk pesticide index ($SPEAR_{pesticides}$) was developed in Europe to evaluate effects of pesticides on benthic macroinvertebrate communities (Liess and Von der Ohe, 2005), and has been applied successfully in several continents (Schäfer et al., 2012). For example, significant correlations have been found between the $SPEAR_{pesticides}$ index and pesticide concentrations in streams in 8 countries in Europe, as well as in Australia and Siberia (r^2 between 0.62 and 0.68) (Schäfer et al., 2012). The $SPEAR_{pesticides}$ index has been shown to respond selectively to pesticide stressors and to be relatively insensitive to most other stressors, although its performance can be affected by severe habitat degradation (siltation and channelization) and low dissolved oxygen (Liess et al., 2008a; Jes Jessen Rasmussen et al., 2011; Schäfer et al., 2011, 2007).

The objectives of the present study were to: (1) evaluate relationships between insecticide concentrations in stream sediments and aquatic invertebrate communities of the Argentine Pampas using the SPEAR index; (2) examine the major changes in invertebrate communities associated with pesticide exposure; and (3) test the applicability of the SPEAR index to invertebrate communities associated with both benthos and emergent vegetation.

Methods and Materials

Study Locations and Sampling Schedule

We carried out the study over a three year period (Dec 2011 – Feb 2014), monitoring 23 sites in small streams located in two regions of the Argentine Pampas, including an intensive soy production region and a mixed agriculture and livestock region (Figure 1; Table S1). In the La Plata region, the principal land use was cattle grazing, with scattered plots of soy production and other agriculture. In the Arrecifes region, intensive soy production was the predominant land use. In the La Plata region, four streams were sampled during two monitoring events in the 2011 to 2012 season only, including four sampling sites in one watershed and the remaining sites in separate watersheds. In the Arrecifes region, 16 sites were sampled over three years (2012-2014), and all sampling sites were on tributaries of the Arrecifes River.

Catchments were delineated using topographical maps to estimate catchment size (Table S2). Substrates in streams of both regions generally consisted of sediment with no rocks and little woody debris, although a few sites in Arrecifes contained some gravel. Stream depths ranged from about 0.6 m to > 2 m (although all except two in the La Plata region were < 1 m), and widths ranged from about 3 m to about 25 m (Table S2). Most streams included emergent (e.g. *Typha* spp. and *Scirpus* spp.) and submerged vegetation (e.g. *Potamogeton*, *Ceratophyllum* and *Egeria*), and many in the La Plata region were also characterized by abundant floating vegetation (e.g. *Eichornia, Lemna* and *Azolla*).

Biological, physico-chemical, and insecticide concentration components involved concurrent stream sampling that was generally timed to occur within a week after a heavy rainfall during or soon after the peak insecticide application period, which usually occurs between December and March. During the 2011-2012 growing season, samples in the La Plata region were collected in December 2011 and March 2012, and in the Arrecifes region in January and March 2012. During 2013 and 2014, samples were collected only in Arrecifes, in February of both years (Table 1).

Physico-chemico, habitat and geographical variables

At each sampling site, pH, conductivity, dissolved oxygen, and temperature were measured with a Yellow Springs Instruments SI 556 multi-parameter probe (Yellow Springs, OH, USA). During 2013 – 2014, turbidity was measured with a portable turbidity meter (Hanna Instruments 93414, Woonsocket, RI, USA). Sediment samples were collected for sediment grain size analysis, and organic carbon analysis by ferrous sulfate titration (USDA 1996) (Table S2).

Water samples for analysis of nutrients and major ions were collected in 1 l bottles and kept in coolers with ice until analyzing or freezing within 24 hours of collection. Water samples were filtered (Whatman GF/C) and suspended solids were measured based on weight of filtered material. Dissolved nutrient and major ion concentrations were determined in the filtered water (APHA 2005). Soluble reactive phosphorus was determined by colorimetry through reaction with molybdate-ascorbic acid; nitrite and nitrate by hydrazine reduction followed by diazotization; and ammonium by the reaction of indophenol blue (APHA 2005). Calcium and magnesium were determined by atomic absorption, sodium and potassium by photometry, bicarbonates by Gran titration, sulfates by turbidimetry, and chlorides by silver nitrate titration (APHA 2005).

At each site visit, maximum stream width and depth were measured. Catchments for each site were delineated in GIS using topographical layers, and catchment areas were calculated. Elevation and stream gradient immediately upstream of each site was estimated based on topographical contours (Table S2). In 2013 – 2014, stream velocity was measured and approximate percent area coverage of emergent, submerged, and floating vegetation was estimated (Table S2).

Sediment sample collection and insecticide analysis

The methods for sediment sample collection and analysis of insecticides have been previously described (Hunt et al., 2016). Briefly, composite sediment samples were prepared from 3 to 5 locations at each site, and insecticides were extracted from sediments by sonication (You et al. 2008). Samples collected in 2011-2012 were analyzed for cypermethrin, lambda-cyahalothrin, endosulfan and chloryrifos by gas chromatography-electron capture detection (GC-ECD). Samples collected in 2013-2014 were analyzed for the same insecticides plus additional pyrethroid and organochlorine insecticides using gas chromatography – mass spectrometry – negative chemical ionization (GC-MS-NCI).

Toxic unit calculation

Insecticide toxic units (TUs) were calculated for all sediment samples:

$$TU = C_i / EC50_i$$

where C_i was the insecticide concentration in sediment normalized for total organic carbon (TOC), and $EC50_i$was the 10-d median effects concentration for mortality or immobilization for each insecticide.

The sediment LC50 values for freshwater aquatic invertebrates were identified for sensitive species (Table 2). Most of the LC50 values used in the present study were for the amphipod *Hyalella azteca*, which is known to be very sensitive to pyrethroids and chlorpyrifos (Weston and Lydy 2010). Although *H. azteca* does not occur in Argentina, several closely related species (*H. curvispina*, *H. pampeana*, and *H. pseudoazteca*) are important components of the aquatic invertebrate communities in the Pampas, and the pesticide sensitivity of *H. curvispina* has been shown to be similar to that of *H. azteca* (Mugni et al., 2013; Hunt, unpublished data). For endosulfan, *Chironomus tentans* was more sensitive than *H. azteca* (You et al., 2004); accordingly, the LC50 for *C. tentans* was used to calculate the TU. Toxicity of pesticides in sediment is highly dependent on organic carbon content; therefore, the concentrations were normalized for total organic carbon to calculate TU values.

TU values for all insecticides were summed to calculate total insecticide TUs, and TU values for all pyrethroid insecticides were used to calculate total pyrethroid TUs. When summing TU values, all insecticides that were detected in the data set were included, assigning a concentration of half the quantification limit for pesticides that were not detected in the sample, or detected below the reporting limit. This approach was used because many of the insecticides were frequently detected below the reporting limit; thus it was known that they were present but could not be accurately quantified (Hunt et al., 2016). The data sets for each sampling event were not an adequate size to use a statistical approach such as maximum likelihood estimate to estimate values of concentrations below the quantification limit (Helsel, 2012). While this approach may overestimate total TU values in some samples, in most cases the insecticides not detected in a sample did not contribute more than 1% of the total TU value. Insecticides that were measured but not detected in the relevant sample group were not included in TU calculations for that sample group.

Macroinvertebrate collection and identification

Benthic macroinvertebrate samples were collected by dragging a 30cm D-frame dip net with 500 µm mesh (Wildco, Yulee, FL, USA) over the bottom sediment of each 1 m transect. Because of high variability in the number of organisms obtained, sample size was adapted over time to ensure a sufficient number of organisms in each sample (Table 1). In 2011-2013, all invertebrates from the entire composite sample (approximately $1.5m^2$ in 2011-2012, $2.7m^2$ in 2013) were sorted and identified. In 2014, a subsampling method was used. A sample of approximately 2.7 m^2 was obtained at each site, and the sample material was homogenized and divided into 24 quadrats. Organisms from randomly selected quadrats were sorted until a total count of 500 organisms per sample was reached, or until organisms from all quadrats were sorted. This is close to the upper range of counts used in US biomonitoring programs involving fixed-numbers of organisms (Carter and Resh, 2013). Once initiating the sorting of a quadrat, it was finished to completion even if the target of 500 organisms was reached before finishing the quadrat.

Macroinvertebrate communities associated with emergent vegetation were sampled only during 2011-2012, and only at sites with sufficient emergent vegetation. Five 1m vegetation transects were swept with a 30cm D-frame dip net (net opening area of approximately 600 cm^2) with 500 µm mesh (Wildco, Yulee, FL, USA), for a total sample area of approximately $1.5m^2$.

All samples were preserved in the field in 80% ethanol, later sieved (500 µm) in the laboratory, sorted under 3X magnification, and identified under a stereoscopic microscope. Insects, hydroids, decapods, and amphipods were generally identified to family or lower level, and other taxa were identified by higher taxonomic groups (oligochaetes, nemerteans, turbellarians, leeches, nematodes, gastropods, bivalves, isopods, ostracods) using keys from Dominguez and Fernandez (2009) and Merritt and Cummins (2008).

SPEAR index and optimization of sensitivity thresholds

The $SPEAR_{pesticides}$ index classifies each taxon as either ‘‘species at risk’’ or ‘‘species not at risk’’ based on four biological traits: (1) physiological sensitivity to organics compounds (2) generation time; (3) pesticide exposure potential; and (4) migration ability (Liess and Ohe, 2005).

In the current version of the $SPEAR_{pesticides}$ index (http://www.systemecology.eu/spearcalc/, Version 0.9.0), binary values are assigned for each trait as follows: (1) physiological sensitivity of 1 for taxa with relative sensitivity > threshold, otherwise 0; (2) generation time sensitivity of 1 for taxa with generation time > = threshold, otherwise 0; (3) exposure sensitivity of 1 for epibenthic taxa, or 0 for sediment-dwelling taxa; and (4) migration sensitivity of 0 for organisms with documented ability to migrate rapidly, 1 for all others. A taxon is defined as ‘‘species at risk’’ only if values for all four traits are equal to 1.

The $SPEAR_{pesticides}$ value for each sample is defined as:

$$\mathrm{SPEAR}_{\mathrm{pesticides}} = \frac{\sum_{i=1}^{n} \log(x_i + 1) \cdot y}{\sum_{i=1}^{n} \log(x_i + 1)} \cdot 100$$

where *n* is the number of taxa, x_i is the abundance of the taxon *i* and *y* is 1 if taxon *i* is classified as “species at risk”, otherwise 0.

Although in the present study some taxa were identified to genus or species level in some samples, they could not consistently be identified to a level lower than family; therefore we used family as the lowest taxonomic level for calculation of $SPEAR_{pesticides}$ values. Some families found in the present study were not included in the existing SPEAR database, which was based primarily on European taxa; for these missing families we assigned the trait values available for higher taxonomic levels.

It is likely that generation times of similar multivoltine taxa in the Pampas are shorter than in most temperate zones because they can reproduce during most of the year. Although reproduction of some taxa in Pampas streams may be greatly reduced during some periods of the year,sufficient data do not exist to identify generation times of local taxa. In addition, the invertebrate community composition of Pampas streams may be different than communities in the temperate streams where the $SPEAR_{pesticides}$ index has been validated. For $SPEAR_{pesticides}$, the default threshold value for physiological sensitivity to pesticides is -0.36 (a taxon must have a relative sensitivity score greater than -0.36 to be considered sensitive). The relative sensitivity score for each taxon is calculated relative to *Daphnia magna* (Von der Ohe and Liess, 2004). The default threshold value for generation time is 0.5 yr (a taxon must have a generation time of at least 0.5 yr to be considered sensitive). These threshold values can be adjusted based on local invertebrate communities. In the present study, we used an optimization approach to adjust the pesticide sensitivity and generation time thresholds using Argentine Pampas data sets.

For each data group A to E (Table 1), we simultaneously optimized the values for the pesticide sensitivity and generation time thresholds to achieve maximum inverse correlation between SPEAR values and log-transformed TU values. Global optimization was performed with the differential evolution algorithm, using the DEoptim package in R (Mullen et al., 2011). To avoid overfitting to data sets with small sample size, optimum threshold values calculated for each of the four benthic invertebrate sample groups (A-D) were averaged to obtain final threshold values applied to all benthic and vegetation-associated invertebrate samples. It is reasonable to expect that the optimum threshold values may be different for vegetation-associated communities than for benthic communities, but there were two few vegetation-associated invertebrate samples to perform optimization without high potential for overfitting.

The default SPEAR exposure potential values are based on exposure in the water column because the $SPEAR_{pesticides}$ index has mostly been related to pesticide concentrations in water. However, insecticides in the present study were measured in sediment, because these insecticides are hydrophobic and more likely to be adsorbed to sediments than to be dissolved in water. Therefore, insecticide exposure to both epibenthic and sediment dwelling organisms is likely to be high. Consequently, as part of the $SPEAR_{pesticides}$ optimization process, we compared the results using default taxa exposure values, and exposure values of 1 (exposed) for all epibenthic and sediment-dwelling taxa.

Additional bioassessment metrics

In addition to the $SPEAR_{pesticides}$ index, we calculated the relative abundance metrics of taxa groups that were selected based on their high abundance in the region, and/or known high sensitivity or tolerance to pesticides and other pollutants (Table 3) (Chang et al., 2014; Rubach et al., 2010). We also calculated the Shannon diversity index and taxa richness. Samples containing more than 300 organisms were rarefied to a constant size of 300 organisms to reduce the effect of sample size (Barbour and Gerritsen, 1996).

Data analysis

We performed regression analysis on three data sets, each of which contained variable values that were averaged over two sampling events (Table 1). For benthic invertebrate samples, we used average values for the two events in 2011-2012, and for the two events in 2013-2014. For vegetation-associated invertebrate samples, we used average values for the two events in 2011-2012.

First, we calculated univariate linear regression relationships (lm function in R version 3.2.2) between insecticide TU values (log transformed) and all response metrics (Table 3). For metrics that were significantly correlated with log TU values, we then performed multiple linear regression to evaluate the relative importance of insecticides and other variables in determining variance in each response metric.

At each site we had measured values for many water quality, habitat, and watershed characteristics (Table S2), many of which were highly correlated. To avoid predictor variables with high collinearity, we used a correlation matrix to select variables that were highly correlated with response variables but not with other predictor variables. After initial variable selection, we checked variance inflation factors (VIFs) with the full model to avoid high collinearity ("vif" function in R package "cor") to confirm that all VIFs for all variables were < 3.

We then selected the best predictive models based on Akaike information criterion values corrected for small sample size (AICc) and p-values. The ΔAICc for each model was calculated as the difference between the AICc for the model and the lowest AICc of all models. For each predictor variable in selected models with ΔAICc < 4 and p-value < 0.05, we determined the magnitude and direction of coefficients using multi-model averaging across selected models (Grueber et al., 2011) using the dredge and model.avg functions in the R package MuMIn version 1.15.1 (Barton, 2015). Relative importance of each predictor variable was calculated as the relativized sum of the Akaike weights over all of the selected models containing the variable of interest (Barton, 2015). Importance ranges from 0 (parameter not given any explanatory weight in any of the selected model) to 1 (parameter included in all selected models).

Results

Insecticide TU values

The most commonly detected insecticides were also those that are reported to be the most heavily used in soy production in Argentina: chlorpyrifos, endosulfan (and its degradation product endosulfan sulfate), cypermethrin, and lambda-cyhalothrin (Table 2). Bifenthrin and organochlorine insecticides were detected only occasionally.

Insecticide detection patterns varied between the two regions. In the mixed agriculture region of La Plata, the maximum total insecticide TU values for the 2011 and 2012 sampling events were 0.66 and 0.14 (based on *C. tentans*&*H. azteca*), and chlorpyrifos was the only insecticide that was consistently detected. In the intensive soy production region of Arrecifes, maximum total insecticide TU values for the four sampling events ranged from 0.51 to 1.36, and multiple insecticides were detected at most locations (Table 2). In Arrecifes, pyrethroid (cypermethrin and lambda-cyhalothrin) concentrations were the primary contributors to high TU values, while in La Plata endosulfan was the primary contributor.

SPEAR$_{pesticides}$ threshold optimization

Based on optimization results, we selected SPEAR$_{pesticides}$ trait sensitivity threshold values to use for the regression analysis. The pesticide sensitivity threshold was optimized at a range between -0.24 and 0.06, and we selected a value of -0.2. The generation time threshold was optimized at a range between 0 and 0.5 year, and we selected a value of 0.5 because this is the threshold value that has been applied in other SPEAR$_{pesticides}$ studies. Applying these threshold values, the only taxa that were considered sensitive with respect to all four traits (overall SPEAR$_{pesticides}$ score of one) were the Trichoptera family Hydroptilidae, and all Crustacea taxa (Hyalellidae, Palaemonidae, Aeglidae, Caridae, Ostracoda). For most data sets, slightly better correlations were achieved when exposure values for all taxa (both epibenthic and sediment dwelling) were set equal to 1 (sensitive with respect to exposure), so we set exposure values equal to 1 for all taxa.

Univariate linear regressions

SPEAR$_{pesticides}$was the metric that performed most consistently well in predicting invertebrate community response to insecticides. SPEAR$_{pesticides}$, % crustaceans and % amphipods were the only response metrics that were significantly correlated with insecticide TUs for more than one data group (Table 3). The only other community metric that exhibited a significant correlation with TU was % bivalvia, which was inversely correlated with pyrethroid TU only for the 2013-2014 benthos data group. SPEAR$_{pesticides}$ was the only response metric that was significantly correlated with total insecticide TU values for all three averaged data sets, consistently showing a trend of decreasing values with increasing TU values (r^2 = 0.35 to 0.42, p-value = 0.001 to 0.03) (Table 3; Figure 2). Percent crustaceans and % amphipods were significantly correlated with total insecticide TU values in two of the three data groups. The relative abundance of both crustaceans and amphipods were highly correlated for all data sets ($0.39 < r^2 < 0.98$; p-value <0.018), and the two metrics performed very similarly (Table 3).

Total insecticide TU was usually a better predictor than pyrethroid TU for the three most responsive metrics (SPEAR$_{pesticides}$, % crustaceans, and % amphipods), with the exception of the 2013-2014 benthos data group. Although significant correlations were obtained between pyrethroid TU and some predictor variables (SPEAR$_{pesticides,}$ % crustaceans and % amphipods), the correlations were weaker than those for total insecticide TU.

Relative importance of variables

Based on univariate results, two response metrics (SPEAR$_{pesticides}$and % crustacea) were selected for multivariate linear regression to determine the relative importance of insecticide TU and non-pesticide parameters. Amphipods were not included in this analysis because their response is very similar to that of total crustacea (Table 3).

For all data sets, total insecticide TU was the most important variable in explaining variance in the SPEAR$_{pesticides}$ index (Table 4). The relative variable importance of total insecticide TU ranged from 0.85 to 1.00 for the three data groups. Relative importance of non-pesticide predictor variables used in models that included total insecticide TU ranged from 0.10 to 0.38.

Chloride was found to be important in explaining variability in relative abundance of crustaceans, but less so for $SPEAR_{pesticides}$. Major ions were measured only in 2011-2012, and many of the eight major ions were found to be collinear with each other, with conductivity, and with TU values. To avoid redundancy, chloride was the only ion selected as a predictor variable in the full models for 2011-2012, because it had low negative correlation with TUs and high correlation with two response metrics ($SPEAR_{pesticides}$ and % crustacea). For the 2013-2014 data set, conductivity was selected instead of chloride, because major ions were not measured. Chloride was identified as a variable with low to high importance in most of the 2011-2012 averaged models (Table 4). In particular, it had high importance in explaining the variance in % crustacea (relative importance 0.57-1.00). Total conductivity was not found to be important in explaining variance of any response metrics for the 2013-2014 data set. For 2011-2012, the models were also run using conductivity in place of chloride to compare their relative importance, and the importance of conductivity was consistently lower than chloride (not shown).

Nutrients did have some influence on the $SPEAR_{pesticides}$ index, but no apparent influence on the relative abundance of crustaceans. Nutrient concentrations were measured during all sampling events, and some nutrients were collinear. Most were also positively correlated with TU values, but not with response metrics. Soluble reactive phosporous (SRP) was selected as a predictor variable in the full models, because it had low correlation with TUs. SRP was identified as a variable with low to moderate importance in the $SPEAR_{pesticides}$ models, but had no importance in explaining variance in % crustacea (Table 4).

Streams size did not appear to influence the $SPEAR_{pesticides}$ index values, but was more important in explaining variability in crustacean abundance. Several metrics were available to represent stream size, including width, depth, elevation, gradient, and catchment area. Depth was selected as a predictor variable in the full models, because it was the stream size variable with lowest correlation with TUs. Depth was identified as an important variable for only one data set (2011-2012 benthos), where it had high importance in explaining % crustacea (Table 4).

Floating vegetation % was the type of vegetation most highly correlated with response metrics, and was included in the full model for 2013-2014 but was not found to be an important predictor for any metric. Vegetation was not included in the 2011-2012 models because no quantitative data were collected.

Discussion

The results of the present study demonstrate that insecticide concentrations in streams of the Argentina Pampas are correlated with changes to aquatic invertebrate community compositions, and that these changes are not highly influenced by other variables included in the study. To our knowledge, this is the first field study that has demonstrated such effects in soy production regions. In intensive soy production regionsinthe midwest region of the United States, as well as in Brazil and Argentina, many studies have reported frequent detections of insecticides, as well as toxicity to specific sensitive species (Casara et al., 2012; Di Marzio et al., 2010; Ding et al., 2010; Hladik and Kuivila, 2012; Jergentz et al., 2004a, 2004b; Laabs et al., 2002; Mugni et al., 2011). However, none of these studies investigated effects on entire invertebrate communities over a gradient of pesticide concentrations.

Studies using the $SPEAR_{pesticides}$in agricultural regions of other countries have found similar results to the present study, usually with stronger correlations (Liess and Von der Ohe, 2005; Schäfer et al., 2012, 2011, 2007). Schäfer et al. (2012) reported that eight studies in Europe, Siberia and Australia found very good correlations between $SPEAR_{pesticides}$and pesticide TU values ($0.62 < r^2 < 0.68$). These studies used life history trait databases based on taxa from the respective regions. In Argentina, and in South America in general, such taxa trait data are currently lacking. Although the $SPEAR_{\mathbf{pesticides}}$ index was developed in Europe, it performed well in the Argentine Pampas with only minor modifications, and would likely improve as more data are obtained on the traits of South American taxa such as generation time and migration rates.

While the relative sensitivity values in the $SPEAR_{pesticides}$ index are based on all toxicity data reported globally (Von der Ohe and Liess, 2004), the vast majority of aquatic toxicity tests were performed on species from Europe and North America (Hagen and Douglas, 2014). Kwok et al. (2007) found that tropical species may be more sensitive than temperate species to pesticides, while temperate species are likely to be more sensitive to metals. The lack of biological trait data for local taxa may be one possible contributor to lower performance of the $SPEAR_{pesticides}$ index in Argentina compared to Europe and Australia, where r^2 values typically ranged from about 0.6 to 0.7 (Schäfer et al., 2011).

Most previous SPEAR studies used lower taxonomic levels than the present study which was based on family level, but it has been demonstrated that the explanatory power of the family level $SPEAR_{pesticides}$ index is not significantly lower than the species level $SPEAR_{pesticides}$ index. Therefore, it is not likely that the taxonomic resolution level is responsible for the somewhat lower correlations found in the Argentine Pampas.

Although most previous studies applying the $SPEAR_{pesticides}$ index have been based on pesticide concentrations measured in stream water, Schafer et. al (2011) found that pesticide concentrations in sediments were more strongly correlated with $SPEAR_{pesticides}$ values in Australian streams. This was surprising given that more compounds and approximately twice the number of total detections above the quantitation limit was found in grab water samples compared to sediment samples. However, the higher detection frequency in water was likely because of more hydrophilic compounds detected in water, and lower quantitation limits in analysis of water samples. In addition, the average toxicity of pesticides in water samples was lower than that for sediment samples (Schäfer et al., 2011). In the present study, the most commonly used insecticides are more likely to partition to sediment than to water, and to remain in sediments at elevated concentrations for at least several weeks after peak water concentrations (Hunt et al., 2016). Therefore, insecticide concentrations in sediment are expected to be a good indicator of invertebrate exposure to insecticides for the present study.

Crustaceans, especially amphipods in the genus *Hyalella*, comprised a large part of the stream invertebrate communities in the Argentine Pampas (Table 3), and also played an important role as sensitive taxa in performance of the $SPEAR_{pesticides}$ index in this region. This is in contrast to the role of amphipods in the $SPEAR_{pesticides}$ index in Europe, where the species *Gammarus pulex* is abundant. Although *G. pulex* has high physiological sensitivity to pesticides, this species can migrate very fast and thus is assigned a $SPEAR_{\mathbf{pesticides}}$ score of 0 (considered not at risk for pesticides)(Liess and Von der Ohe, 2005). We were unable to find data on the migration rate of

Hyalella species, but the decrease in relative abundance of amphipods corresponding with increase in insecticide toxic units demonstrates that *Hyalella* should be considered to have overall sensitivity to insecticides. The influence of chloride content on crustacean abundance is supported by previous studies, although data are limited. Crustaceans and other freshwater organisms are known to osmoregulate hypertonically by active transport of ions into the hemolymph, and chloride is the principal inorganic anion in the hemolymph of crustaceans (Soucek and Kennedy, 2005). Low chloride concentrations may limit distrubution of some euryhaline amphipods such as Hyalella spp, and chlorides have been found to have a protective effect on sulfate toxicity to Hyalella azteca (Soucek and Kennedy, 2005). Freshwater amphipods of both the Hyalellidae and Gammaridae families are traditionally considers to be shredders of leaf litter, although recent studies have shown plasticity in trophic levels and functional roles (Acosta and Prat, 2011). Although *Hyalella* usually was the most abundant crustacean in most samples collected in the present study, at some sites the decapod family Palaemonidae was the only crustacean present, sometimes with very high relative abundance.

This was the first study using the $SPEAR_{pesticides}$ index to evaluate effects of pesticides on invertebrate samples collected from aquatic vegetation, and its performance was similar to that for to benthic invertebrate communities. The data set for vegetation-associated invertebrate communities in the present study was limited, and additional studies would be need to optimize the $SPEAR_{\mathbf{pesticides}}$ index specifically for this type of sample. However, in the present study the taxa composition (relative abundance of major orders) for vegetation-associated communities was similar to that of benthic communities (Table 3), so it is reasonable to expect that the thresholds would be similar for both communities.

The results of this study are consistent with previous studies that have shown the $SPEAR_{pesticides}$ index to not be highly influenced by non-pesticide variables (Beketov and Liess, 2008; Liess et al., 2008a, 2008b). Beketov and Liess (2008) investigated factors that affect $SPEAR_{organics}$, which uses the same taxa relative sensitivity values as $SPEAR_{pesticides,}$ but does not consider the additional three traits that are included in $SPEAR_{pesticides}$. They showed that $SPEAR_{organics}$ is independent of stream longitudinal gradient, including factors such as altitude, temperature, stream width, nutrients, and velocity. In contrast, metrics such as EPT richness and Shannon diversity were highly correlated with longitudinal factors (Beketov and Liess, 2008). In a study of 24 Australian stream sites, Schäfer et al. (2011) found that pesticide contamination was the only measured variable explaining variation in $SPEAR_{pesticides}$. Other measured variables included many water quality, habitat and landscape variables.

Some previous studies did identify specific non-pesticide variables that can affect performance of the $SPEAR_{pesticides}$ index. Rasmussen et al. (2011) investigated 212 streams in Denmark, where they found that $SPEAR_{pesticides}$ values were negatively correlated with ortho-phosphate concentrations, BOD and macrophyte coverage. The present study also found that phosphorus (SRP) helped to explain variance in $SPEAR_{pesticides}$, but was much less important than insecticide TUs. While the present study did not find macrophyte coverage to be important, we measured this variable only in the 2013-2014 data group. We selected only sites that consistently had high dissolved oxygen levels, so BOD is unlikely to be a confounding factor. Bunzel et al. (2014) analyzed data from 663 stream sites in central Germany and reported that $SPEAR_{\mathbf{pesticides}}$ values decreased with with increasing hydromorphological degradation, especially in sites with concrete channels or straight artificial stream beds. In the present study we selected sites that were not highly channelized, so this is not likely to be an important confounding factor in our analysis.

Conclusions

This study established a correlation between insecticide TUs in stream sediments and changes in aquatic invertebrate communities of the Argentine Pampas. The $SPEAR_{pesticides}$ index consistently showed a significant decrease with increasing insecticide TUs, across all data groups. For all data sets evaluated, insecticide TU was the most important variable in explaining variability in the $SPEAR_{pesticides}$ index, indicating that it is relatively insensitive to non-pesticide stressors.

The $SPEAR_{pesticides}$ index performed equally well for aquatic invertebrate communities associated with emergent vegetation as it did for benthic invertebrate communities. This was true even though the $SPEAR_{\mathbf{pesticides}}$ thresholds were optimized for benthic invertebrate communities.

The most dramatic effect in invertebrate communities was seen on the crustaceans, which are highly sensitive to most insecticides. Crustaceans, especially amphipods in the genus *Hyalella*, comprised a large part of the stream invertebrate communities in the Argentine Pampas (Table 3), and also played an important role as sensitive taxa in performance of the $SPEAR_{pesticides}$ index.As shredders, amphipods play an important role in leaf litter decomposition, and large reductions in their abundance may impact influence ecosystem functioning.

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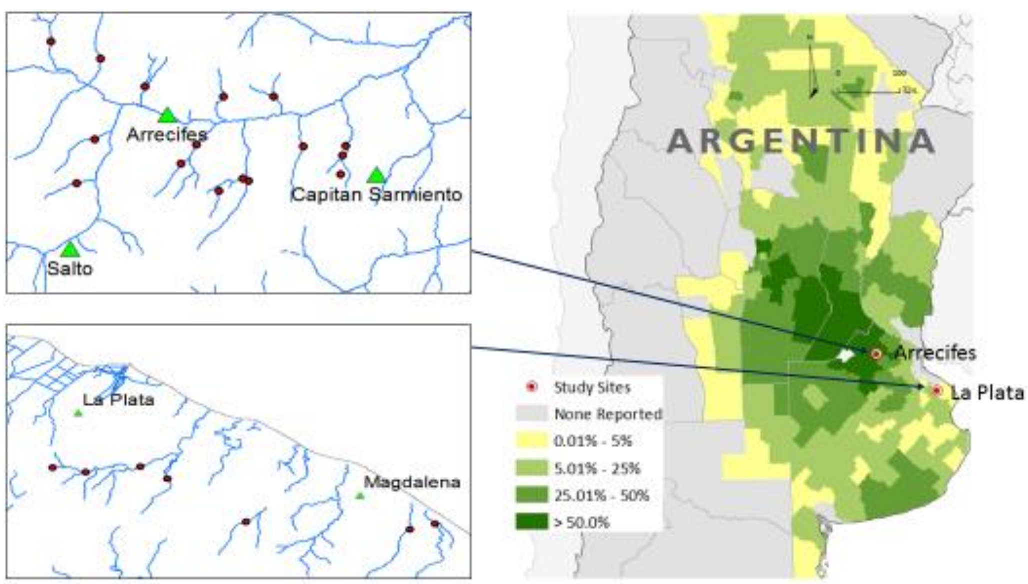

Figure 1. Overview of study regions and soy production intensity in Argentina, and stream sampling locations in the La Plata and Arrecifes regions. Soy production intensity as percent of total land use by province based on government reported data: http://www.minagri.gob.ar.

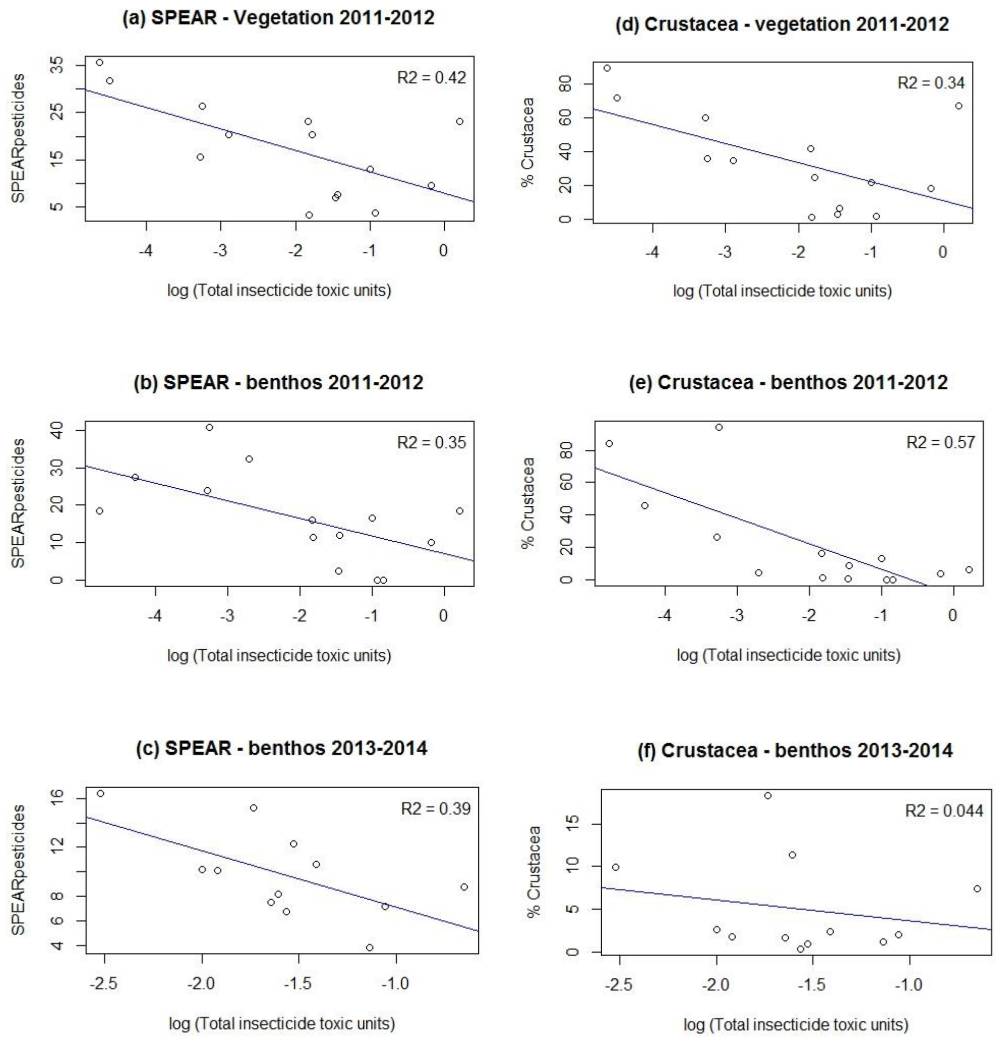

Figure 2. Univariate linear regression between total insecticide TUs and community metrics for (a) $SPEAR_{pesticide}$ values of emergent vegetation samples in La Plata and Arrecifes with values averaged over two sampling events in 2011-2012), (b) $SPEAR_{pesticide}$ values of benthos samples in La Plata and Arrecifes with values averaged over two sampling events in 2011-2012, (c) benthos samples in Arrecifes with values averaged over two sampling events in 2013-2014, (d) % crustacea values of emergent vegetation samples in La Plata and Arrecifes with values averaged over two sampling events in 2011-2012), (e) % crustacea values of benthos samples in La Plata and Arrecifes with values averaged over two sampling events in 2011-2012, and (f) % crustacea of benthos samples in Arrecifes with values averaged over two sampling events in 2013-2014.

Table 1. Invertebrate sampling methods and sample groups for SPEAR threshold optimization and regression analysis.

Group	Date	Region	SPEAR Threshold Optimization	Regression Analysis (values averaged over 2 dates)
Benthos Samples				
A	Dec 2011 Jan 2012	La Plata (n = 7) Arrecifes (n = 5)	n = 12	n = 14[a]
B	March 2012	La Plata (n = 5) Arrecifes (n = 7)	n = 12	
C	Feb 2013	Arrecifes (n = 12)	n = 12	n = 12[b]
D	Feb 2014	Arrecifes (n = 12)	n = 12	
Emergent Vegetation Samples				
E	Dec 2011 Jan 2012	La Plata (n = 7) Arrecifes (n = 5)		n = 14[c]
F	March 2012	La Plata (n = 6) Arrecifes (n = 6)		

[a] 10 sites with values averaged over 2 dates (Groups A and B), 4 sites sampled only once during 2011-2012.

[b] 12 sites with values averaged over 2 dates (Groups C and D)

[c] 11 sites with values averaged over 2 dates (Groups E and F), 3 sites sampled only once

Table 2. Detection frequencies and maximum toxic units (TUs) for each sampling event, for insecticides that had at least one TU value >0.01. TUs were calculated as the ratio of the carbon-normalized concentration in sediment over the carbon-normalized LC50. Insecticide concentrations were reported in Hunt et al. (2016).

			La Plata		Arrecifes			
Pesticide	LC50 (ng/g organic carbon)	Statistic	Dec 2011	Mar 2012	Jan 2012	Mar 2012	Feb 2013	Feb 2014
Chlorpyrifos	4160[a]	Max TU	0.01	0.02	0.06	0.16	0.09	0.08
		Frequency[b]	29%	57%	86%	100%	100%	67%
Endosulfan	960[c]	Max TU	0.32	0.04	0.14	0.18	0.01	0.09
		Frequency[b]	29%	14%	57%	43%	8%	17%
End.sulfate	5220[c]	Max TU	0.28	0.07	0.08	0.10	0.12	0.03
		Frequency[b]	29%	14%	29%	57%	58%	33%

Cypermethrin	380[a]	Max TU	0.05	nd	1.15	0.97	0.38	0.13
		Frequency[b]	29%	0%	29%	29%	33%	8%
L-cyhalothrin	450[a]	Max TU	NA	0.02	NA	0.71	0.23	0.16
		Frequency[b]		0%		29%	17%	0%
Bifenthrin	520[d]	Max TU	NA	NA	NA	NA	nd	0.36
		Frequency[b]					0%	17%
Total pyrethroid TU [e,g]		Max TU	0.05	0.05	1.15	1.16	0.45	0.41
Total insecticide TU [f,g]		Max TU	0.66	0.14	1.23	1.36	0.51	0.54

[a] LC50 for *Hyalella azteca* from Weston et al. 2013

[b] Frequency of detection above the highest quantitation limit of 0.5 ng/g dw in sediment.

[c] LC50 for *Chironomus tentans* from You et al. 2005

[d] Frequency of detection above the highest quantitation limit of 0.25 ng/g dw in sediment

[e] Total pyrethroid TU values for each sample were calculated by summing the TU values for each pyrethroid.

[f] Total insecticide TU values for each sample were calculated by summing the TU values for each insecticide.

[g] A concentration value of half the quantitation limit was assigned for pesticides detected in the sample group but not detected in the sample, or detected <QL in the sample.

Table 3. Optimized $SPEAR_{pesticides}$ thresholds, relative taxa abundance, and univariate regression results.

Response Variable			Univariate Regression Results						
			Data Group	Total Insecticide TU			Pyrethroid TU		
				Trend[a]	r^2	p-value	Trend[a]	r^2	p-value
Benthic invertebrate communities									
Optimized SPEAR Thresholds									
	Sensitivity	Generation Time (yr)							
$SPEAR_{pesticides}$	-0.2	0.5	2011-12	+	0.35	0.03		0.10	0.28
			2013-14	+	0.39	0.03	+	0.58	0.004
	Community Metrics mean (min – max)								
	La Plata	Arrecifes							
Richness	6.6	12	2011-12		0.24	0.07		0.14	0.19
	(2-15)	(3-26)	2013-14		0.09	0.36		0.02	0.68
Diversity	0.90	1.4	2011-12		0.14	0.19		0.05	0.46
	(0.26-2.2)	(0.37-2.5)	2013-14		0.10	0.31		0.05	0.47

% crustacea	32	6	2011-12	+	0.57	0.002	+	0.35	0.03
	(0-96)	(0-35)	2013-14		0.04	0.51		0.09	0.33
% amphipoda	16	5	2011-12	+	0.53	0.003	+	0.36	0.02
	(0-83)	(0-33)	2013-14		0.04	0.51		0.09	0.34
%ephemeroptera	1.3	23	2011-12		0.00	0.74		0.04	0.49
	(0-9.0)	(0-83)	2013-14		0.01	0.73		0.01	0.74
% trichoptera	0	1.1	2011-12[b]		-	-		-	-
	(0-0)	(0-16)	2013-14		0.21	0.14		0.15	0.22
% EPT	1.3	24	2011-12		0.00	0.74		0.04	0.49
	(0-9.0)	(0-83)	2013-14		0.00	0.88		0.00	0.86
% diptera	3.1	20	2011-12		0.20	0.11		0.25	0.07
	(0-15)	(0-89)	2013-14		0.00	0.99		0.00	0.97
% chironomidae	2.6	19	2011-12		0.22	0.09		0.27	0.06
	(0-15)	(0-83)	2013-14		0.00	0.99		0.00	0.95
% oligochaetes	40	16	2011-12		0.20	0.11		0.01	0.70
	(0-91)	(0-83)	2013-14		0.00	0.87		0.02	0.70
% bivalvia	2	4	2011-12		0.08	0.32		0.05	0.46
	(0-12)	(0-38)	2013-14		0.32	0.05	-	0.47	0.01
% gastropoda	4	16	2011-12		0.01	0.78		0.04	0.49
	(0-17)	(0-89)	2013-14		0.01	0.72		0.04	0.54
Vegetation-associated invertebrate communities									
	Optimized SPEAR Thresholds[c]								
	Sensitivity	Generation Time (yr)							
$SPEAR_{pesticides}$	-0.2	0.5	2011-12	+	0.42	0.01		0.14	0.19
	Community Metrics mean (min – max)								
	La Plata	Arrecifes							
Richness	13(4.3-25)	18(5.3-30)	2011-12		0.05	0.42		0.09	0.28
Diversity	1.4(0.3-2)	1.7(0.8-2.9)	2011-12		0.17	0.14		0.15	0.17

% crustaceans	41 (0-94)	25 (0.1-68)	2011-12	+	0.34	0.03		0.07	0.37
% amphipods	40 (0-94)	23 (0.1-67)	2011-12	+	0.29	0.04		0.06	0.39
%ephemeroptera	0.7 (0-3.1)	19 (0-70)	2011-12		0.2	0.60		0.03	0.56
% trichoptera	0.2 (0-2.1)	0.1 (0-0.8)	2011-12		0.02	0.62		0.00	0.82
% EPT	0.9 (0-5.2)	19 (0-71)	2011-12		0.02	0.60		0.03	0.57
% diptera	4.7 (0-42)	12 (0.3-34)	2011-12		0.03	0.56		0.07	0.37
% chironomids	1.3 (0-5.5)	7.9(0.2-23)	2011-12		0.11	0.25		0.13	0.21
% oligochaetes	14 (0-71)	16 (0-63)	2011-12		0.18	0.13		0.05	0.46
% bivalvia	0.1 (0-0.7)	0.1 (0-0.7)	2011-12		0.02	0.62		0.00	0.82
% gastropoda	6.5 (0-36)	5.9 (0-34)	2011-12		0.17	0.13		0.00	0.79

[a] "+" signifies positive significant correlation, "-" signifies negative significant correlation

[b]No trichoptera were present in benthic samples collected in 2011-2012.

[c] Thresholds optimized for benthic samples were applied to vegetation samples, because too few vegetation data sets were available to optimize thresholds.

Table 4. Averaged model results and relative importance of predictor variables

Period	Sampling Matrix	Response Metrics	Averaged Models			Relative Variable Importance			
			adj r^2	AICc	p-value	Total TU	SRP	Chloride[a]	Depth
2011-2012	Benthos	$SPEAR_{pesticides}$	0.29-0.38	≤111.6	0.03	1.00	0.38	-	-
		% Crustacea	0.80	≤-2.4	≤0.0002	0.12	-	1.00	1.00
	Vegetation	$SPEAR_{pesticides}$	0.24-0.38	≤107.6	≤0.042	0.85	0.16	0.27	-
		% Crustacea	0.28-0.31	≤8.6	≤0.0499	0.57	-	0.57	-
			Averaged Models			**Relative Variable Importance**			
			adj r^2	AICc	p-value	Total TU	SRP	Conductivity[a]	Depth
2013-2014	Benthos	$SPEAR_{pesticides}$	0.33-0.40	≤68.7		1.00	0.27	-	-
		% Crustacea [b]	-	-	-	-	-	-	-

[a]Major ions were measured only in 2011-2012, and for this period chloride was found to have higher importance than conductivity in explaining variance in response metrics.

[b] No models with p-value < 0.5were found

Supplementary Information

Table S1. Sampling Sites and Schedule

Site Name	Latitude	Longitude	Dec-11	Jan-12	Mar-12	Feb-13	Feb-14
La Plata (Mixed agriculture/ grazing)							
Remes	35 1 31.87S	57 59 39.6W	X		X		
Poblet	35 2 2.45S	57 56 34.3W	X		X		
Pescado	35 1 23.97S	57 51 27.42W	X		X		
Cajaravilla	35 4 6.37S	57 48 57.17W	X		X		
Blanco	35 8 30.23S	57 26 23.98W	X		X		
Destino	35 8 15.35S	57 23 41.21W	X		X		
Arregui	35 7 38.83S	57 41 39.01W	X		X		
Arrecifes (Intensive soy production)							
H0	34 8 31.58S	59 50 31.74W		X	X		
H1	34 10 6.13S	59 49 57.32W		X			
H2	34 10 19.46S	59 50 42.60W		X	X		
H5	34 734.67S	59 50 14.31W				X	X
A3	34 10 56.82S	59 58 56.13W			X		
A1	34 7 28.59S	60 3 30.76W		X	X		
A2	34 10 42.52S	59 59 23.43W			X		X
Tres Horquetas	34 2 52.40S	59 56 40.00W				X	X
Canete	34 1 53.64S	60 8 5.50W		X	X	X	X
Contador 2	34 9 20.13S	60 4 51.35W				X	X
Gomez	34 7 38.72S	59 54 1.50W		X		X	X
Helves 2	34 2 53.30S	60 0 56.71W				X	X
Las Animas	34 6 59.39S	60 12 32.90W				X	X
Los Ingleses	33 59 10.67S	60 11 59.21W				X	X
Luna 2	34 11 54.76S	60 1 31.78W					X
Maguire	33 55 19.70S	60 16 5.90W		X	X	X	X
Salto 2	34 11 11.90S	60 14 7.22W				X	X

Table S2. Summary statistics of site characteristics in each region

		La Plata	Arrecifes
Maximum depth (m)	minimum	0.20	0.25
	maximum	>2	0.80
	median	0.50	0.60
	mean	0.62	0.56
	standard deviation	0.26	0.12
Maximum width (m)	minimum	0.6	3.0
	maximum	25.0	8.0
	median	10.0	5.0
	mean	11.8	5.7
	standard deviation	7.6	1.5
Maximum velocity (m/s) [a]	minimum		0.06
	maximum		0.85
	median		0.34
	mean		0.36
	standard deviation		0.20
Estimated flow (m3/s) [a]	minimum		0.04
	maximum		0.69
	median		0.15
	mean		0.21
	standard deviation		0.17
Gradient (%)	minimum	0.1	0.1
	maximum	1.7	0.5
	median	0.4	0.3
	mean	0.6	0.3
	standard deviation	0.5	0.1
Elevation (m)	minimum	5.0	30.0
	maximum	53.0	50.0
	median	29.0	48.8
	mean	26.8	43.1
	standard deviation	19.2	8.7

Catchment size (Ha)	minimum	1259	1824
	maximum	28163	28659
	median	6607	4124
	mean	9046	6790
	standard deviation	7860	7258
Water conductivity (uS/cm)	minimum	220	663
	maximum	4000	1796
	median	921	895
	mean	906	961
	standard deviation	813	247
Water pH	minimum	6.7	7.0
	maximum	9.0	9.1
	median	7.4	8.0
	mean	7.7	7.9
	standard deviation	0.7	0.5
Dissolved oxygen (mg/L)	minimum	4.00	4.90
	maximum	12.90	18.77
	median	9.30	10.27
	mean	8.48	10.78
	standard deviation	2.6	3.2
Water turbidity (NTU) [a]	minimum		3.9
	maximum		96.0
	median		14.7
	mean		21.9
	standard deviation		22.2
% sediment TOC	minimum	2%	0.26
	maximum	12%	2.00
	median	10%	1.16
	mean	8%	1.16
	standard deviation	4%	0.52
% sediment fines (silt and clay) [a]	minimum		52.7
	maximum		78.1

	median	64.7
	mean	65.5
	standard deviation	8.6
% emergent vegetation coverage [a]	minimum	0.0
	maximum	25.0
	median	2.5
	mean	6.0
	standard deviation	8.6
% submerged vegetation coverage [a]	minimum	1.0
	maximum	90.0
	median	20.0
	mean	34.1
	standard deviation	32.6
% floating vegetation coverage [a]	minimum	0.0
	maximum	20.0
	median	0.0
	mean	2.0
	standard deviation	5.7

[a]Measured only in 2013-2014.

Table S3. Relative abundance and detection frequency of benthic and vegetation-associated invertebrate taxa in the La Plata and Arrecifes study streams.

	Benthos				Emergent Vegetation			
	La Plata		Arrecifes		La Plata		Arrecifes	
	Relative Abundance (%)	% Samples Detected	Relative Abundance (%)	% Samples Detected	Relative Abundance (%)	% Samples Detected	Relative Abundance (%)	% Samples Detected
Coleoptera								
Chrysomelidae	0.000	0.0	0.000	0.0	0.020	16.7	0.267	45.5
Curculionidae	0.021	8.3	0.003	0.0	0.273	58.3	0.089	45.5
Dryopidae	0.000	0.0	0.000	0.0	0.010	8.3	0.000	0.0
Dytiscidae	0.000	0.0	0.073	0.0	0.151	25.0	0.153	36.4
Elmidae	0.000	0.0	1.696	83.3	0.000	0.0	0.025	18.2
Georissidae	0.000	0.0	0.000	0.0	0.000	0.0	0.013	9.1
Haliplidae	0.000	0.0	0.000	0.0	0.030	16.7	0.051	9.1
Heteroceridae	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
Hydrophilidae	0.145	16.7	0.132	8.3	0.626	66.7	1.921	81.8
Noteridae	0.021	8.3	0.126	0.0	0.040	16.7	0.127	36.4
Scarabaeidae	0.000	0.0	0.003	0.0	0.000	0.0	0.000	0.0
Scirtidae	0.000	0.0	0.015	0.0	0.020	0.0	0.140	54.5
Sphaeriusidae	0.000	0.0	0.003	0.0	0.000	0.0	0.000	0.0
Staphylinidae	0.000	0.0	0.000	0.0	0.030	16.7	0.153	45.5
Diptera								
Ceratopogonidae	0.000	0.0	0.389	66.7	0.000	0.0	0.763	72.7
Chironomidae	2.305	50.0	17.797	100.0	0.384	50.0	3.663	100.0
Culicidae	0.042	8.3	0.006	0.0	0.262	16.7	0.038	9.1
Empididae	0.000	0.0	0.024	16.7	0.000	0.0	0.000	0.0
Ephydidrae	0.000	0.0	0.392	8.3	0.010	0.0	0.407	9.1
Muscidae	0.000	0.0	0.012	0.0	0.000	0.0	0.000	0.0
Psychodidae	0.000	0.0	0.000	0.0	0.000	0.0	0.013	9.1
Sarcophagidae	0.000	0.0	0.000	0.0	0.000	0.0	0.025	18.2
Simuliidae	0.000	0.0	0.144	41.7	0.000	0.0	0.000	0.0
Stratiomyidae	0.062	16.7	0.083	0.0	0.081	25.0	0.407	45.5
Syrphidae	0.000	0.0	0.003	0.0	0.000	0.0	0.000	0.0
Tabanidae	0.000	0.0	0.000	0.0	0.000	0.0	0.025	18.2
Ephemeroptera								
Baetidae	0.000	0.0	31.138	41.7	0.061	25.0	16.840	45.5
Caenidae	1.225	33.3	5.258	91.7	0.273	41.7	2.277	63.6

	Benthos				Emergent Vegetation			
	La Plata		Arrecifes		La Plata		Arrecifes	
	Relative Abundance (%)	% Samples Detected	Relative Abundance (%)	% Samples Detected	Relative Abundance (%)	% Samples Detected	Relative Abundance (%)	% Samples Detected
Leptohyphidae	0.000	0.0	0.827	33.3	0.000	0.0	0.025	18.2
Hemiptera								
Belostomatidae	0.000	0.0	0.046	0.0	0.313	50.0	0.420	81.8
Corixidae	0.083	25.0	0.006	0.0	0.232	25.0	0.038	18.2
Gerridae	0.000	0.0	0.003	0.0	0.000	0.0	0.509	36.4
Hebridae	0.000	0.0	0.015	0.0	0.010	0.0	0.000	0.0
Helotrephidae	0.000	0.0	0.003	0.0	0.000	0.0	0.051	18.2
Hydrometridae	0.000	0.0	0.000	0.0	0.030	16.7	0.025	18.2
Macroveliidae	0.000	0.0	0.000	0.0	0.000	0.0	0.038	27.3
Mesoveliidae	0.021	8.3	0.003	0.0	0.030	16.7	0.331	27.3
Naucoridae	0.000	0.0	0.006	0.0	0.010	8.3	0.064	27.3
Nepidae	0.021	8.3	0.000	0.0	0.000	0.0	0.013	9.1
Notonectidae	0.768	16.7	0.003	0.0	0.828	50.0	0.178	27.3
Pleidae	0.000	0.0	0.083	0.0	0.030	8.3	0.254	36.4
Veliidae	0.000	0.0	0.000	0.0	0.000	0.0	0.089	45.5
Lepidoptera								
Crambidae	0.000	0.0	0.080	25.0	0.010	8.3	0.000	0.0
Noctuidae	0.000	0.0	0.003	0.0	0.000	0.0	0.000	0.0
Odonata								
Aeshnidae	0.042	8.3	0.000	0.0	0.202	33.3	0.025	18.2
Calopterygidae	0.000	0.0	0.043	8.3	0.000	0.0	0.000	0.0
Gomphidae	0.000	0.0	0.000	0.0	0.010	8.3	0.000	0.0
Libellulidae	0.000	0.0	0.012	0.0	0.061	25.0	0.280	9.1
Coenagrionidae/ Protoneuridae	0.228	25.0	3.454	25.0	1.504	66.7	1.717	90.9
Trichoptera								
Hydroptilidae	0.000	0.0	0.202	41.7	0.071	8.3	0.038	9.1
Hydropsychidae	0.000	0.0	0.478	41.7	0.000	0.0	0.000	0.0
Acari	0.042	16.7	0.285	66.7	0.040	16.7	0.064	18.2
Bivalvia	6.956	16.7	5.135	83.3	0.010	8.3	0.038	18.2
Gastropoda	0.664	91.7	15.668	100.0	1.252	91.7	4.236	63.6
Turbellaria	0.021	8.3	0.876	75.0	2.876	41.7	1.806	63.6
Hirudinea	4.755	66.7	0.937	83.3	8.639	83.3	7.644	54.5

Nematoda	0.000	0.0	0.251	66.7	0.000	0.0	0.000	0.0
Nemertea	0.000	0.0	0.119	16.7	0.000	0.0	0.000	0.0
Oligochaeta	65.365	58.3	6.801	91.7	4.824	50.0	11.002	45.5
Hydridae	0.000	0.0	0.006	0.0	0.000	0.0	0.000	0.0
Isopoda	0.000	0.0	0.000	0.0	0.182	16.7	0.000	0.0
Decopoda								
Palaemonidae	8.181	33.3	0.101	8.3	1.070	33.3	0.445	27.3
Aeglidae	0.000	0.0	0.058	33.3	0.000	0.0	0.000	0.0
Ostracoda	0.145	16.7	0.640	50.0	0.040	16.7	0.509	36.4
Amphipoda								
Hyalellidae	8.887	50.0	6.559	66.7	75.454	58.3	42.763	100.0

CHAPTER 5

Agricultural stressors affecting invertebrate communities in Atlantic Forest streams, and mitigation effectiveness of forested riparian buffers

Agricultural stressors affecting invertebrate communities in South American Atlantic Forest streams, and mitigation effectiveness of forested riparian buffers

Abstract

We investigated the influence and relative importance of insecticides and other agricultural stressors in determining variability in invertebrate communities in small streams in intensive soy production regions of Brazil and Paraguay. In Paraguay, 17 sites were sampled over two seasons (January and December 2013), and all sampling sites were on tributaries of the Pirapó River in the state of Itapúa. In Brazil, 18 sites were sampled once in November 2013, and all sampling sites were on tributaries of the San Francisco River in the state of Paraná.The riparian buffer zones generally contained native Atlantic forest remnants and/or introduced tree species at various stages of growth. Although minimum buffer width in Brazilian streams was negatively correlated with insecticide concentrations and buffer width was found to have moderate importance in mitigating effects on some sensitive taxa such as mayflies, insecticides had low relative importance in explaining variability in invertebrate communities. Paraguay and Brazil both have laws requiring forested riparian buffers, and the fact that almost all streams had ample forested riparian buffer zones is likely to have mitigated the effects of pesticides on stream invertebrate communities in these regions.In Brazilian streams, the percent coverage of soft depositional sediment in streams was the most important agriculture-related explanatory variable, and the overall stream habitat score was the most important variable in Paraguay streams.

Introduction

In recent years, soybean production has become a major export crop for multiple countries in South America, raising concern about environmental impacts. Between 1995 and 2011, soy cultivation area expanded by 126% in Brazil (Castanheira and Freire 2013). In Paraguay, soycultivation area increased from 1.3 Mha in 2000-2001 to 2 Mha in 2007-2008 (Garcia-Lopez and Arizpe 2010). Land use changes caused by this expansion of soy cultivation are likely to have multiple adverse environmental effects, including reductions in ecosystem complexity, loss of biodiversity, deforestation, increased erosion, adverse effects of agrochemicals, and increased greenhouse gas emissions (Botta et al. 2011; Castanheira and Freire 2013; Lathuilliere et al. 2014).

Conversion of land to intensive agriculture can result in degradation of adjacent streams and stream ecosystem through impacts such as nutrient enrichment, sedimentation, pesticide toxicity, and deforestation (Gücker et al., 2009; Jones et al., 2001; Matthaei et al., 2010). For example, in headwater streams of the Brazilian Cerrado, agricultural streams had higher nutrients, reduced channel morphology, higher velocities, lower microbial biomass, and lower community respiration compared to less disturbed streams (Gücker et al., 2009). Moreover, in a multiple-watershed study that evaluated influence of landscape variables on sediment and nutrient load, amount of agriculture explained 50% of the variation in total nitrate concentrations (Jones et al., 2001).

Agriculture adjacent to streams can adversely impact benthic macroinvertebrate communities through multiple mechanisms. Agriculture-related stressors can include habitat degradation (e.g.

loss of cover, deposition of fine sediments), hydrological modification (e.g. channelization, less diversity in pool/run/riffle regimes) and impacts to water quality (e.g. pesticide toxicity, nutrient eutrophication, increased turbidity and conductivity) (Matthaei et al., 2010; Stehle and Schulz, 2015; Stone et al., 2005; Whiles et al., 2000). Moreover, pesticides used in agriculture can have severe impacts on stream water quality and ecosystems, and the insecticides used in soy production in South America are known to be especially toxic to aquatic invertebrates (Hunt et al., 2016; Mugni et al., 2011). A recent meta-analysis of 838 studies across 73 countries found that over 50% of measured insecticide concentrations in water bodies exceeded regulatory threshold levels for surface waters or sediments (Stehle and Schulz, 2015), and another analysis of data from Europe and Australia reported that pesticides reduced both species and family richness of aquatic invertebrate communities (Beketov et al., 2013).

As a management strategy, stream buffer width may be one of the most important factors in mitigating transport of pesticides, sediment,and other pollutants to streams in agricultural areas (Bunzel et al., 2014; Jones et al., 2001; Rasmussen et al., 2011; Stone et al., 2005), and recent regulations in both Brazil and Paraguay require forested riparian buffer zones. For example, in Paraguay, Resolution 485/03 by the Ministry of Agriculture requires a protected zone of 100 m around all water bodies. In Brazil, a new forest code was approved in 2012 (Law No.12.651/12) establishing that riparian buffer zone requirements should vary with the general use of the land adjacent to the water body, the aquatic environment, the stream width, and the size of the rural property. As a general rule for stream widths of 10 m or less, the legislation requires a buffer width of 15 m of native riparian forest in rural areas or 30 m if in areas newly converted for rural activities.

The objectives of the present study were to evaluate: (1) the relative importance of pesticides and other agriculture-related stressors in explaining variation in invertebrate community metrics in Atlantic forest streams; and (2) the effectiveness of forested riparian buffer zones in mitigating adverse effects on streamsof this region.

Study Locations and Sampling Schedule

The study sites included small streams that flowed through agricultural fields in two intensive soy production regions in the former Atlantic forest habitat of Brazil and Paraguay (Figure 1). In Paraguay, 17 sites were sampled over two seasons (January and December 2013), and all sampling sites were on tributaries of the Pirapó River in the state of Itapúa. In Brazil, 18 sites were sampled once in November 2013, and all sampling sites were on tributaries of the San Francisco River in the state of Paraná. Both study watersheds were on tributaries of the Paraná River.

Streams selected for the present study were not artificially channelized, and had a minimum buffer strip width of at least 3 m between the stream and the adjacent crop fields. The riparian buffer zones generally contained native Atlantic forest remnants and/or introduced tree species at various stages of growth. Stream depths ranged from 0.12m to 0.81m, and widths ranged from 2m to 8.5m (Table 1; Table S2).

Stream sampling was timed to occur during or soon after peak insecticide application periods, which varied depending on planting time. For example, soy can either be planted as an early season crop or a late season crop. The early season crop was generally planted in September or October and harvested in January. The late season crop was typically planted between December and February and harvested several months later. Peak insecticide applications for soy production usually occurred in November and December.

Physico-chemico, habitat and geographical variables

At each sampling site, pH, conductivity, dissolved oxygen, and temperature were measured during each sampling event with a Yellow Springs Instruments SI 556 multi-parameter probe (Yellow Springs, OH, USA). Turbidity was measured with a portable turbidity meter (Hanna Instruments 93414, Woonsocket, RI, USA). Field test kits were used to measure concentrations of ammonium/ammonia (Sera, Germany), ortho-phosphate (CHEMets K-8510, Midland, VA, USA), and nitrate nitrogen (LaMotte 3354-01, Chestertown, MD). Sediment samples were collected for sediment grain size analysis, and organic carbon analysis by ferrous sulfate titration (USDA 1996).

At each site visit, maximum stream width and depth were measured, and maximum and average water velocities were measured with a current meter (Global Water FP311, College Station, TX, USA). Habitat quality was assessed at each site according to the USEPA Rapid Bioassessment Protocol (Barbour et al., 1999) and assigned a score on a scale of 0 to 200. Minimum buffer widths were measured immediately upstream of sampling sites, and confirmed with LANDSAT images. Catchments were delineated in GIS using topographical contours to estimate catchment size, and the percent forest and percent agriculture within each catchment were estimated using LANDSAT images. Elevation and stream gradient immediately upstream of each site was estimated based on topographical contours.

Sediment sample collection and insecticide analysis

The methods for sediment sample collection and analysis of insecticides have been previously described (Chapter 2). Briefly, composite sediment samples were prepared from 3 to 5 locations at each site, and insecticides were extracted from sediments by sonication (You et al., 2008). Samples were analyzed for pyrethroid insecticides, organochlorinated insecticides, and the organophosphate insecticide chloryrifos by either gas chromatography-electron capture detection (GC-ECD) or gas chromatography – mass spectrometry – negative chemical ionization (GC-MS-NCI).

Toxic unit calculation

Insecticide toxic units (TUs) were calculated for all sediment samples:

$$TU = C_i / EC50_i$$

where C_i was the insecticide concentration in sediment normalized for total organic carbon (TOC), and $EC50_i$was the 10-d median effects concentration (LC50) for each insecticide.

The sediment LC50 values for freshwater aquatic invertebrates were identified for sensitive species (Table 2; Chapter 2). Most of the LC50 values used in the present study were for the amphipod *Hyalella azteca*, which is known to be very sensitive to pyrethroids and chlorpyrifos (Weston and Lydy 2010). Although *H. azteca* does not occur in Brazil or Paraguay, the closely related *H. curvispina* complex occurs throughout South America (Dominguez and Fernandez, 2009), and the pesticide sensitivity of *H. curvispina* has been shown to be similar to that of *H. azteca* (Mugni et al., 2013; Hunt, unpublished data). For endosulfan, the LC50 for the more sensitive *Chironomus tentans* was used to calculate TUs, because it is substantially lower than the LC50 for *H. azteca* (You et al., 2004). Toxicity of pesticides in sediment is highly dependent on organic carbon content; therefore, the concentrations were normalized for total organic carbon to calculate TU values.

TU values for all insecticides were summed to calculate total insecticide TUs, and TU values for all pyrethroid insecticides were used to calculate total pyrethroid TUs. When summing TU values, all insecticides that were detected in the data set were included, assigning a concentration of half the quantification limit for pesticides that were not detected in the sample, or detected below the reporting limit. Insecticides that were measured but not detected in the sample group were not included in TU calculation.

Macroinvertebrate collection and identification

Benthic macroinvertebrate samples were collected by kick-sampling with a 30cm D-frame dip net with 500 µm mesh (Wildco, Yulee, FL, USA). With each net placement, the substrate was disturbed approximately 0.5m upstream of the net. For the first sampling event in Paraguay, three kick samples, each collected for a period of 30 s, were composited from each site, and all invertebrates from the composite sample were sorted and identified. At four sites, six additional 30s kick samples were collected several days later because of very low organism counts in the first sampling event. For subsequent sampling events in Paraguay and Brazil, sample size was increase to ensure a sufficient number of organisms in each sample, and a subsampling method was used. A larger sample was obtained at each site (30 kick samples, each collected for a period of 20 s), and the sample material was homogenized and divided into 24 quadrats. Organisms from randomly selected quadrats were sorted until a total count of 500 organisms per sample was reached, or until organisms from all quadrats were sorted. This is close to the upper range of counts used in US biomonitoring programs involving fixed-numbers of organisms (Carter and Resh, 2013). Once initiating the sorting of a quadrat, it was finished to completion even if the target of 500 organisms was reached before finishing the quadrat.

All samples were preserved in the field in 80% ethanol, later sieved (500 µm) in the laboratory, sorted under 3X magnification, and identified under a stereoscopic microscope. Insects, decapods and amphipods were generally identified to family, genus, or species level, and other taxa were identified by higher taxonomic groups (oligochaetes, nemerteas, turbellarias, leeches, nematodes, gastropods, bivalves) using keys from Dominguez and Fernandez (2009) and Merritt and Cummins (2008).

SPEAR$_{pesticides}$ index

The Species at Risk pesticide index (SPEAR$_{pesticides}$) was developed in Europe to evaluate effects of pesticides on benthic macroinvertebrate communities(Liess and Ohe, 2005), and has been applied successfully in several continents (Schäfer et al., 2012). We recently applied the SPEAR$_{pesticides}$ index in streams located in soy production regions of Argentina, and found that it performed reasonably well (r^2 = 0.35 to 0.42) with only minor modifications consisting of adjusting the sensitivity thresholds for life history traits (Chapter 4).

The $\text{SPEAR}_{\text{pesticides}}$ index classifies each taxon as either ‘‘species at risk’’ or ‘‘species not at risk’’ based on four biological traits: (1) physiological sensitivity to toxicants; (2) generation time; (3) pesticide exposure potential; and (4) migration ability (Liess and Ohe, 2005). In the current version of the $\text{SPEAR}_{\text{pesticides}}$ index (http://www.systemecology.eu/spearcalc/, Version 0.9.0), binary values are assigned for each trait as follows: (1) physiological sensitivity of 1 for taxa with relative sensitivity > threshold, otherwise 0; (2) generation time sensitivity of 1 for taxa with generation time > = threshold, otherwise 0; (3) exposure sensitivity of 1 for epibenthic taxa, or 0 for sediment-dwelling taxa; and (4) migration sensitivity of 0 for organisms with documented ability to migrate rapidly, 1 for all others. A taxon is defined as ‘‘species at risk’’ only if values for all four traits are equal to 1.

The $\text{SPEAR}_{\text{pesticides}}$ value for each sample is defined as:

$$\text{SPEAR}_{\text{pesticides}} = \frac{\sum_{i=1}^{n} \log(x_i + 1) \cdot y}{\sum_{i=1}^{n} \log(x_i + 1)} \cdot 100$$

where n is the number of taxa, x_i is the abundance of the taxon i and y is 1 if taxon i is classified as “species at risk”, otherwise 0.

Generation times for each taxon in the established SPEAR database had been previously identified based on European trait databases (Schäfer et al., 2007). Because generation times of similar multivoltine taxa in the subtropical Atlantic Forest are likely to be shorter than in temperate zones, they likely can reproduce during all seasons; however, sufficient data do not exist to identify generation times of local taxa. In addition, the invertebrate community composition of Atlantic Forest streams is different than communities in the temperate streams where the SPEAR index has been validated. For $\text{SPEAR}_{\text{pesticides}}$, the default threshold value for physiological sensitivity to pesticides is -0.36 (a taxon must have a relative sensitivity score greater than -0.36 to be considered sensitive). The default threshold value for generation time is 0.5 yr (a taxon must have a generation time of at least 0.5 yr to be considered sensitive). These threshold values can be adjusted based on local invertebrate communities.

In the present study, we applied two versions of the $\text{SPEAR}_{\text{pesticides}}$index: the European version with default trait threshold values and described above; and another version with trait threshold values that have been optimized for Argentine Pampas invertebrate communities (see also Chapter 4). Although we attempted to use the same approach to optimize the trait threshold values for the Atlantic Forest invertebrate communities, it was not successful because a significant univariate correlation between insecticide TU values and $\text{SPEAR}_{\text{pesticides}}$ values could not be achieved with the data sets obtained in the present study.

Although in the present study some taxa were identified to genus or species level in some samples, they could not consistently be identified to a level lower than family. Therefore we used family as the lowest taxonomic level for calculation of $\text{SPEAR}_{\text{pesticides}}$ values. Some families found in the present study were not included in the existing SPEAR database which was based primarily on European taxa; for these missing families we assigned the trait values available for higher taxonomic levels.

Additional bioassessment metrics

In addition to the $SPEAR_{pesticides}$ index, we calculated the relative abundance metrics of taxa groups that were selected based on their common occurrence in the region, and/or known high sensitivity or tolerance to pesticides and other pollutants (Table S3) (Chang et al., 2014; Rubach et al., 2010). We also calculated metrics that are used by the local environmental protection agency in Toledo, Brazil including modified Biological Monitoring Working Party (BMWP) scores and average score per taxon (BMWP ASPT) (Daniel Buss, personal communication). Other metrics we calculated included total taxa density per m^2, relative abundance of three most dominant taxa,Shannon-Weaver diversity index,total taxa richness, coleoptera family richness, trichoptera family richness, and EPT family richness (Table S3). Samples containing more than 300 organisms were rarefied to a constant size of 300 organisms to reduce the effect of sample size (Barbour and Gerritsen, 1996).

Data analysis

We used several statistical methods to evaluate the relationships between environmental variables and invertebrate communities. Because riparian buffer zones are expected to have a protective effect on invertebrate communities through multiple mechanisms, we first evaluated relationship of buffer width with habitat metrics and water and sediment quality parameters. We then used univariate regression to evaluate the performance of the indices, and finally applied multiple regression analysis to evaluate the relative importance of each predictor in explaining invertebrate community metrics. All data analyses were carried out in the open-source statistical software R version 3.2.2(R Development Core Team, 2015).

Principle component analysis (PCA) ordination was used to examine patterns in environmental variables for the two study regions and for the high and low buffer width groups. Environmental parameters examined in the PCA included all variables in Table S2, with the exception of water pH, temperature, and dissolved oxygen. pH was not included because of little variation, and DO and temperature were not included because they depend in part on the time of day that sampling was conducted.The R function prcomp was used to carry out the PCA, and data for all variables were centered and scaled prior to analysis.

Non-metric multi-dimensional scaling (NMDS) ordination was used to visually examine patterns in community structure and environmental variables for the two study regions, and for high and low buffer width groups (>50m and <50m). The function metaMDS in the vegan package in R was used to carry out the NMDS ordination, using Bray-Curtis distance. All taxa counts were square root transformed, and data were standardized using Wisconsin double standardization.

We then used the BIO-ENV procedure (Clarke and Ainsworth, 1993) to identify the subset of environmental variables that best explain the variation in community composition. Because the NMDS analysis indicated that community structure was distinct for each of the two regions, we did this analysis separately for each region. BIO-ENV finds the optimum correlation between a community dissimilarity matrix and multiple environmental dissimilarity matrices, with all possible combinations of environmental variables. For the community matrix, we used Bray-Curtis distance, and for the environmental variable matrix we used Euclidean distance. For Paraguay sampling sites, when environmental variable values were measured during both sampling events, average values were used, and invertebrate data collected during two sampling events at the same site were combined. NMDS, correlation analyses, and BIO-ENV were carried out using the vegan package in R (Oksanen et al., 2013).

Multiple regression analysis was conducted for each of the 31 invertebrate community metrics (Table S3). Because the NMDS analysis indicated that community structure was distinct for each of the two regions, we performed the analysis separately for the Paraguay and Brazil data sets, and variable values for Paraguay were averaged over the two sampling events.For the full models, we selected the parameters that are both likely to be affected by adjacent agriculture, and to have an effect on invertebrate communities. Thse parameters included mainly habitat, substrate, and sediment quality predictor variables (RBP score, % riffles, %TOC in sediment, % large woody debris (LWD), % fine particulate organic matter (FPOM), % course particulate organic matter (CPOM), % soft depositional sediment coverage, total insecticide TU (log transformed) and % sediment fines (only in Brazil because sufficient data were not available for all sites in Paraguay). Turbidity was also included, but other water quality parameters (conductivity, pH, temperature) were not included, either because they did not vary much within regions or depended on the time of day sampled. To evaluate the effect of land use and buffer width, we also included % agriculture and buffer width as predictor variables, and to account for the effect of watershed characteristics we included catchment size and elevation.

To avoid using predictor variables with high collinearity, we used a correlation matrix to select variables that were highly correlated with response variables but not with other predictor variables. After initial variable selection, we checked variance inflation factors (VIFs) with the full model to avoid high collinearity ("vif" function in R package "cor"). All predictor variables had VIF values less than three.

We then selected the best predictive models based on Akaike information criterion values corrected for small sample size (AICc) and p-values (Barton, 2015). The ΔAICc for each model was calculated as the difference between the AICc for the model and the lowest AICc of all models. For each predictor variable in selected models with $\Delta AICc < 4$ and $p\text{-value} < 0.05$, we determined the magnitude and direction of coefficients using multi-model averaging across selected models (Grueber et al., 2011) using the dredge and model.avg functions in the R package MuMIn version 1.15.1 (Barton, 2015). Relative importance of each predictor variable was calculated as the relativized sum of the Akaike weights over all of the selected models containing the variable of interest (Barton, 2015). Importance ranged from 0 (i.e. parameter not given any explanatory weight in any of the selected model) to 1 (i.e. parameter included in all selected models).

We then used simple linear regressions to analyze the effect of agriculture and buffer width on each of the habitat, substrate, and sediment and water quality parameters that were shown to have high importance in invertebrate community response metrics.

Results

Insecticides Present

In both regions, the most commonly detected insecticide was chlorpyrifos, followed by the pyrethroids cypermethrin, lambda-cyhalothrin, bifenthrin, cyfluthrin, deltamethrin, esfenvalerate and permethrin (Table 2). Although chlorpyrifos was detected in most samples, it is less potent than the pyrethroid insecticides, and the maximum TU values for chlorpyrifos were lower than the total pyrethroid TU values. Endosulfan and its degradate endosulfan sulfate were detected occasionally, but with relatively low TU values. The banned organochlorine insecticides DDT (and degradates), endrin, chlordane, and aldrin were also detected in some samples, but TU values were always below 0.01. The total insecticide and total pyrethroid TU values were highly correlated, and were similar between the two regions(Hunt et al. 2016).

Water and sediment quality

The PCA ordinationshowed that the Paraguay and Brazil sites were clearly distinct on the horizontal axis with respect to certain water quality and sediment quality parameters (organic carbon, % fine sediment, conductivity), but similar with respect to others (insecticide TU values, % soft sediment, and turbidity) (Figure 2a, d). Generally, percent fine sediments and organic carbon were higher in Brazil than in Paraguay (Table S2), and conductivity was higher in Paraguay than in Brazil (Table S2).

The low and high buffer groups were partially overlapping, but slightly separated with regard to parameters on the vertical axis (insecticide TU values, turbidity, and to a lesser extent, % fines and soft sediment) (Figure 2b, d). When split into high and low buffer zone groups by region, the low and high groups were still distinct from each other within each region (Figure 2c), indicating that the differences in pesticide TU values, % soft sediment, and turbidity are more likely to be related to differences in buffer width than to differences between the two regions.

The nutrient results indicated that nutrient concentrations were in the lowest concentration categories (ammonia/ammonium < 0.5 ppm, nitrates < 1 ppm, ortho-phosphate < 0.1 ppm) for all or most sites in both regions. Four sites in Brazil and five sites in Paraguay had nitrate concentrations of 1-2 ppm, and two sites in Brazil had ortho-phosphate concentrations between 0.1-0.2 ppm. Because nutrients concentrations were generally low and did not vary much between sites, nutrients were not included as variables in the statistical analyses.

Habitat quality and landscape characteristics

The PCA ordination showed that the Brazil and Paraguay sampling sites were distinct with respect to certain habitat quality parameters along the vertical axis (Figure 3a, d). Generally, Brazil sites tended to have higher particulate organic matter and large woody debris, and more riffles, while Paraguay sites tended to have a higher percentage of forest in catchment areas, larger buffer zones, and more bedrock and runs in streams (Figure 3, Table 1). However, the overall RBP habitat scores were similar for both regions (Table 1).

The low and high buffer groups were also clearly distinct from each other with regard to the same parameters on the vertical axis that separated the regions (Figure 3b, d). However, when split into low and high buffer zone groups by region, the buffer groups within each region overlapped with each other (Figure 3c), indicating that the differences in habitat quality were primarily an artifact of the differences in the regional data sets, and not a result of buffer size.

In addition to the differences in habitat parameters, Brazil and Paraguay sampling sites were also distinct with respect to catchment landscape characteristics. Catchment sizes of the Paraguay sites tended to be larger than those in Brazil, hence the stream widths and depths were also generally larger. Elevations of all sites in Brazil were higher than those at Paraguay sites, and stream gradients tended to be slightly higher in Brazil but were not significantly different between regions (Table 1).

Invertebrate community analysis

Invertebrate community composition was similar in the two regions, but there were some differences in relative abundance of certain groups, and in families present (Table S4; Figure 4). In Paraguay samples, we identified a total of 49 insect families, including 13 families which were not present in samples collected from Brazil sites (Table S4). In Brazil samples, we identified a total of 38 insect families, two of which were not present in samples collected from Paraguay sites. All of the insect families that were found in one region but not the other occurred only rarely in the region where they were detected (< 1% relative abundance). In Paraguay, the only decapod found was Trichodactylidae, and in Brazil the only decapod found was Aeglidae. No ostracods were found in Paraguay samples, but they were found in very small numbers at two sites in Brazil.

In both regions, the dominant family was Chironomidae (Diptera), but relative abundance was substantially higher in Brazil (Table S3). The second most dominant family in both regions was Elmidae (Coleoptera), and in this case relative abundance was higher in Paraguay. In Paraguay, Leptohyphidae was the most common Ephemeroptera family, while in Brazil, Leptophlebiidae was most common. In Paraguay, trichopterans had higher relative abundance and more families present than in Brazil. Plecopterans were rare in both regions.

The NMDS results indicated that the invertebrate communities were distinct between Brazil and Paraguay (Figure 4a), but not between low and high buffer groups (Figure 4b). However, the stress values for the NMDS analysis done in two dimensions was somewhat high for the Brazil data set (0.20) as well as the combined Brazil and Paraguay data set (0.23), indicating that the results should be interpreted only for identifying rough patterns. For the Paraguay data set, stress was lower (0.13).

The BIO-ENV analysis indicated that for the Paraguay data set, only two variables were important in explaining variation in invertebrate communities: stream width and RBP habitat quality score ($r = 0.42$). However, for the Brazil data set, the maximum correlation between community and environmental dissimilarity matrices ($r=0.39$) was obtained when 10 variables were included in the model: conductivity, turbidity, % soft sediment, % bedrock, % CPOM, buffer width, site elevation, stream gradient, stream width, and stream depth.

Community metrics and relative importance of predictor variables

In both regions, multiple significant models (p-value < 0.05) were selected for four community metrics: %EPT, %EPT-HB, % ephemeroptera and % trichoptera. In Brazil, one additional metric (coleoptera richness) had significant models, and in Paraguay nine additional metrics had significant models (Table 3).

In both regions, % agriculture had moderate to high importance (>0.4) and significant effect (average p-value < 0.05) on %EPT, with a negative influence (Table 3). In Brazil, % agriculture also significantly and adversely affected % Ephemeroptera, but in Paraguay, % agriculture had no importance in explaining variability in % Ephemeroptera. In Brazil, % agriculture had a negative relationship with %EPT-HB, % Trichoptera, and Coleoptera richness, but with low importance (< 0.4) and average p-value > 0.05. In Paraguay, % agriculture had a negative relationship with the ASPT index, with moderate importance (0.46), but average p-value > 0.05.

In Brazil, buffer width appeared to have a mitigating effect, but this relationship was not discernable in Paraguay where most of the sites had mimimum buffer width of 100m or greater. Buffer width had a positive influence and moderate importance on both %EPT-HB and % ephemeroptera in Brazil, but was significant (average p-value > 0.05) only for % Ephemeroptera. In Paraguay, buffer width exhibited little or no importance on all response metrics evaluated (Table 3).

Insecticide TU values did not appear to have an explanatory role for the $SPEAR_{pesticides}$ index or other invertebrate community metrics in either region. In Brazil, insecticide TU had little or no importance for all response metrics. In Paraguay, insecticide TU did have high importance and a significant relationship for two metrics (% Ephemeroptera and the ASPT index), but the positive direction was opposite to the expected relationship. The $SPEAR_{pesticides}$ index values were not significantly correlated with insecticide TU values in either region. In Paraguay, several habitat parameters were the only predictor variables that were important in explaining variability in $SPEAR_{pesticides}$ index values. In Brazil, none of the parameters included in the model were significantly correlated with $SPEAR_{pesticides}$ index values.

Overall, RBP habitat score was the most important parameter in Paraguay, but was not a good explanatory variable in Brazil. In Paraguay, RBP habitat score had high importance and was significant for eight response metrics, always in the expected direction (positive coefficient for seven sensitive response metrics, and negative for relative abundance of the dominant taxon). However, RBP habitat score had no significant effect (average p-value < 0.05) for any response metric in Brazil, and coefficients for three sensitive response metrics were negative (the opposite direction as expected).

With regard to substrate and water quality parameters, % soft depositional sediment coverage was the best predictor variable in Brazil, but its influence in Paraguay was mixed. In Brazil, this parameter had a significant negative effect with moderate to high importance for three response metrics. However, in Paraguay, while the effect was usually as expected (negative for sensitive response metrics %EPT-HB and SPEAR, and positive for relative abundance of three most dominant taxa) it was not as expected for one metric (positive relationship with rare and sensitive taxa). Several other parameters (% riffles, turbidity, course and fine particulate matter, large woody debris, and sediment TOC) had moderate to high importance and were significant for some response metrics, but effects were not always consistent within or between regions (Table 3).

Although adjacent agriculture would be expected to result in increased deposition of soft sediment in streams and degradation of habitat quality, the effect of % agriculture on % soft sediment coverage and on RBP habitat score was not statistically significant in either region. Similarly, there was no statistically significant relationship between buffer width and % soft sediment coverage or RBP habitat score, although the relationship in Paraguay was close to significant (p = 0.053) for soft sediment coverage.

With respect to landscape parameters, catchment area was most important in Brazil, and stream gradient was most important in Paraguay. Generally, catchment area showed a positive relationship with sensitive metrics, while gradient had a negative relationship with sensitive metrics and positive relationship with relative abundance of dominant taxa. In Brazil, catchment size was the most important predictor variable in explaining variability in %EPT-HB and % ephemeroptera.

Discussion

Relative importance of agricultural stressors

While percent of soft sediment coverage had high importance in determining variability in multiple community metrics in both of our study regions, the RBP habitat score was important only in the Paraguay region. The RBP habitat score was also one of only two parameters determined to be important in determining community structure in Paraguay by the BIO-ENV analysis. When we examined each of the 10 parameters that comprise the total RBP habitat score, we found that for the Paraguay data set, the parameters that contributed most variation to the total score were primarily those related to substrate, including embeddedness, sediment deposition, and riffle frequency. Bank stability also contributed highly, and generally corresponded with sediment deposition and embeddedness. There was little variation in scores for flow and channel alteration, as the study streams were generally not altered.

Our study's findings that stressors relating to stream substrate were the most important in determining invertebrate community structure correspond with results fromprevious studies (Richards et al., 1993; Wood and Armitage, 1997). For example, Richards et al. (1993) found that the most important morphological factors affecting invertebrate communities in streams in an agriculture-dominated region were related to substrate composition and fine sediment distribution. In addition to changing the type of substrate available to invertebrates, deposition of fine sediment can alter stream morphology and decrease structural diversity of stream habitat by filling pools and covering hard surfaces such as rocks (Richards et al., 1993; Wood and Armitage, 1997).

None of the analyses conducted in the present study indicated that insecticides were important in determining invertebrate community structure in either region. Insecticide TU levels were not determined to be important either in the BIO-ENV analysis, or in the multiple regression analysis using various bioassessment metrics. The $SPEAR_{pesticides}$ index has been shown to be a sensitive indicator of pesticide effects on invertebrate communities in many different regions of the world (Schäfer et al., 2012), but was not correlated with insecticide levels in the present study. Unlike other bioassessment indices, the $SPEAR_{pesticides}$ index has been shown to respond selectively to pesticide stressors and to be relatively insensitive to most other stressors, although its performance can be affected by severe habitat degradation (siltation and channelization) and low dissolved oxygen (Liess and Ohe, 2005; Jes Jessen Rasmussen et al., 2011; Schäfer et al., 2011, 2007).

There are several factors that may help explain why $SPEAR_{pesticides}$ index values were not significantly correlated with insecticide TU values in either of the Atlantic forest regions we studied. First, the presence of forested riparian buffers along their entire lengths of all streams is likely to increase the resilience and recovery ability of invertebrate communities. Second, the sensitive taxa groups found in the study streams are likely to be sensitive to many habitat and water quality variables in addition to pesticides, potentially confounding the analysis. Third, the $SPEAR_{pesticides}$ index may not work well in tropical and subtropical environments without significant adaptation.

There is ample evidence that forested headwaters provide reservoirs of invertebrate populations that assist in the recovery of downstream communities after disturbance from pesticide exposure (Liess and Ohe, 2005; Orlinskiy et al., 2015). For example, a study in an agricultural region of Germany found that upstream forested headwaters mitigated the effects of pesticides on downstream invertebrate populations (Orlinskiy et al., 2015). In streams in intensive soy production regions of Argentina, we found similar upper levels of insecticides as in the Brazil and Paraguay study regions, but in Argentina the insecticides were more clearly correlated with impacts on sensitive invertebrate taxa (Chapter 4). A possible reason for the difference may be that the stream buffers in Argentina were much smaller and were not forested, thus the invertebrate communities were not as resilient.

In contrast to the Atlantic Forest streams included in the present study, invertebrate communities in streams of the Argentine Pampas tend to contain large percentages of amphipods, which are very sensitive to pesticides but relatively tolerant of many habitat and water quality parameters (Chapters 3 and 4). When the SPEAR index threshold values were optimized for Argentine Pampas streams, only the crustaceans and trichopterans were considered sensitive to pesticides, and amphipods were the most abundant sensitive organisms, making them very important in the performance of the $SPEAR_{pesticides}$ index. In the Atlantic Forest streams, amphipods and other crustaceans were rare, and other sensitive and abundant taxa (such as EPT taxa) tend to be sensitive not only to pesticides but also to many habitat and water quality parameters (Bunzel et al., 2013).

The $SPEAR_{pesticides}$ index has not yet been validated for tropical or subtropical streams. A study in tropical streams of Costa Rica did not find a correlation between pesticide toxic units and a modified version of SPEAR (Rasmussen et al., 2016). However, that study measured only three sites, and all sites suffered from low dissolved oxygen and lacked sensitive taxa. The inconclusive finding with SPEAR may be a result of the study design, including factors such as low number of sampling sites, narrow environmental gradient, and many confounding factors. Although it is possible that the $SPEAR_{pesticides}$ index may not work well in tropical or subtropical environments without significant modification, more study is necessary to determine this.

Influence of forested riparian buffers

The present study's findings in Brazil corroborate findings from other studies that have found riparian buffer zones to be important in mitigating transport of pesticides to streams (Rasmussen et al. 2011; Di Marzio 2010; Bunzel et al. 2014; Reichenberger et al. 2007; Aguiar et al., 2015). Another study of Atlantic Forest streams in Brazil found no pesticides except the herbicide atrazine in streams with a forested buffer of approximately 60m, while multiple pesticides including chlorpyrifos and lambda-cyhalothrin were detected in streams with 12m and 36m of forested buffer width (Aguiar et al., 2015). Buffers comprised of grass or shrubs were not as effective in pesticide removal as forested buffers (Aguiar et al., 2015). Many factors could affect the buffer width needed to protect streams from pesticide exposure, including gradient, type of vegetation, soil properties, types of pesticides applied, timing and amount of pesticides applied, and presence of tile drains or drainage ditches that short-circuit the buffer zones (Reichenberger et al. 2007; Bunzel et al. 2014).

The lack of variation in minimum buffer width at the Paraguay sampling sites likely limited a firm conclusion regarding the influence of buffer width in this region. Approximately half of the Paraguay sites had minimum buffer widths of 100m, which was the minimum required by law. In contrast, in the Brazil study region where buffer width did not exhibit the same clustered pattern, it was the most important variable in explaining insecticide TU values and also had moderate importance in explaining the variability in several invertebrate community metrics. In Chapter 2 I reported that highest insecticide toxic units in both study regions occurred when buffer zone widths were 20 m or less. Moreover, a multiple regression for the Brazil data set indicated that buffer width was the predictor variable that had the greatest influence on total insecticide TU. The selected model included the following predictor variables: buffer width, percent total organic carbon in sediment, and stream gradient ($r^2 = 0.54$; p-value = 0.009). The analysis of relative importance indicated that buffer width contributed 74% of the explained variance in total insecticide TU values, with percent total organic carbon and stream gradient contributing 9 and 17 %, respectively.

Because almost all streams in both regions we studied had relatively large forested stream buffers, it is possible that relative effects of buffer widths in protecting invertebrate communities were less evident in this study than in other similar studies in which streams generally had much smaller buffer width. We measured minimum buffer width observed immediately upstream of each sample site, and confirmed the measurements using LANDSAT images. However, for most streams, the average upstream riparian buffer width was considerably larger than the minimum width measured near the sampling site, and forested buffers typically extended throughout the entire watershed, even around the small headwater streams. Therefore, the streams in the two regions included in the present study can be considered well protected in comparison to streams in many intensive agricultural regions. In contrast, previous studies that have found riparian buffer zones to be effective in mitigating effects on stream invertebrate communities have generally evaluated streams with much smaller protected buffer zones than those in the present study. For example, Whiles et al. (2000) found that land use within 18m of streams in agricultural areas of Nebraska was correlated with invertebrate bioassessment scores. Another study in Ontario reported that forested area within a 30m riparian zone was positively correlated with increases in EPT taxa and taxa diversity (Rios and Bailey, 2006). In our Brazil study area, where buffer widths were generally lower than those in Paraguay, buffer width did have moderate importance in explaining variability in several invertebrate metrics, while in Paraguay it had little or no importance. It is possible that the higher taxa richness observed in Paraguay streams may be due at least in part to the larger riparian buffer widths compared to the Brazil streams.

Although regulation of pesticide mitigation measures often focuses on application practices, landscape level mitigation measures, such as requiring riparian buffer zones, may be easier to implement and enforce. Bereswill et al. (2014) reviewed the efficacy and practicality of risk mitigation measures for diffuse pesticide entry into aquatic ecosystems, and ranked riparian buffer strips as highly effective for mitigating both spray drift and runoff, with high acceptability and feasibility. However, the implementation and enforcement of new riparian buffer requirements in Brazil has been difficult and controversial, especially in regions with small-scale production where a significant amount of a landowner's productive farmland could be lost with compliance (Alvez et al. 2012).

Conclusions

This study did not find a correlation between the $SPEAR_{pesticides}$ index or other bioassessment metrics and insecticide TU values in the Atlantic forest streams included in the study. However, the fact that almost all streams had ample forested riparian buffer zones is likely to have mitigated the effects of pesticides on stream invertebrate communities. The $SPEAR_{pesticides}$ index may still prove to be a useful tool in Atlantic Forest streams without much of a riparian buffer, especially if wider ranges of pesticide concentrations occur.

Results of this study also indicated that non-pesticide agricultural stressors were more important than insecticides in affecting invertebrate communities. In particular, the amount of soft depositional sediment had high importance in explaining variability in several invertebrate community metrics in both Paraguay and Brazil, and the RBP habitat score was very important in explaining variability in multiple metrics in Paraguay.

Although results indicated that riparian buffer width was a moderately important predictor variable in Brazil, but had low importance in Paraguay, it is likely that the findings in Paraguay were limited by the lack of variation in minimum buffer width in that region. More study is necessary to determine the relationship between buffer width and the health of stream invertebrate communities in Atlantic forests.

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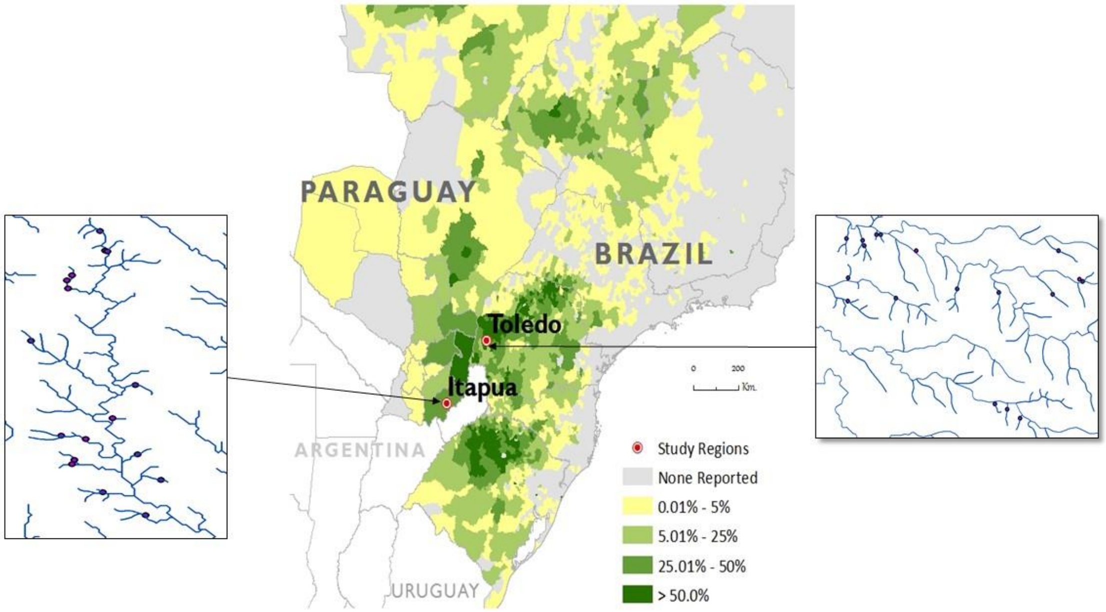

Figure 1. Overview of study regions and soy production intensity in Brazil and Paraguay and sampling locations on tributaries of Pirapó River in Itapua, Paraguay and San Francisco River in Parana, Brazil.

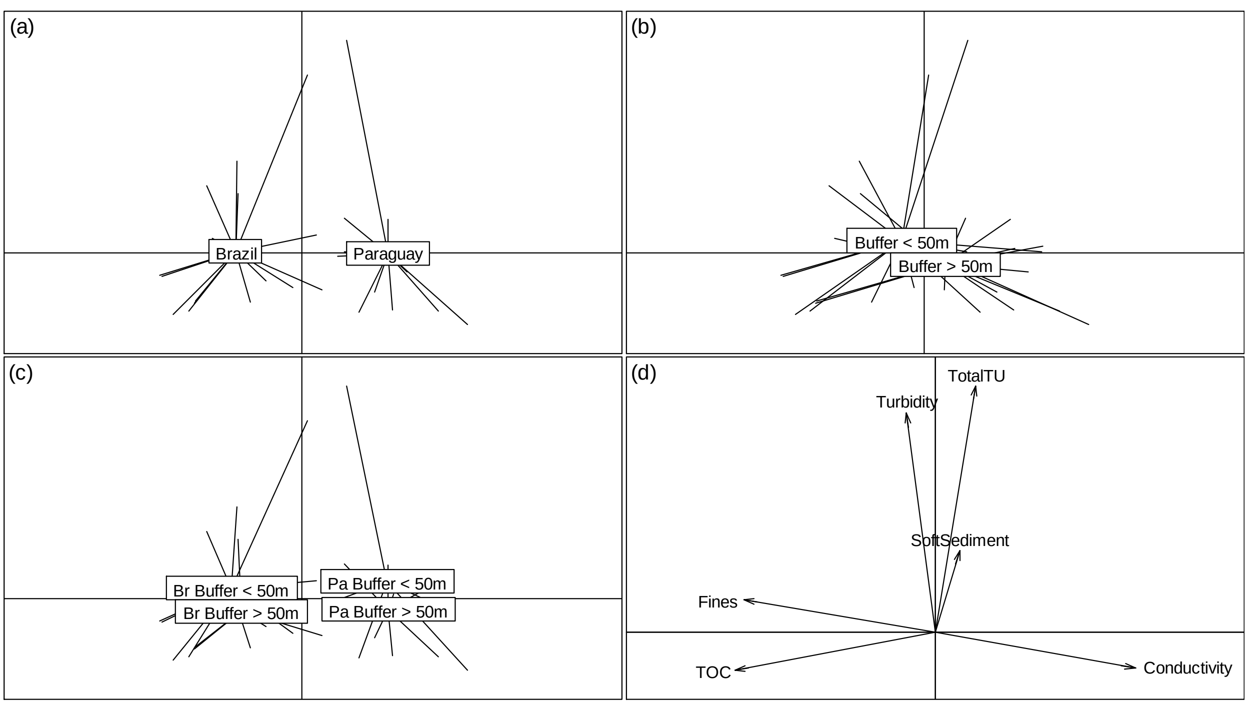

Figure 2. PCA ordination of water and sediment quality parameters for sampling sites in the two study regions (Brazil and Paraguay), the high and low buffer width groups (b), and the high and low buffer width groups within each region (c). For Paraguay sites that were sampled twice, parameter values were averaged.

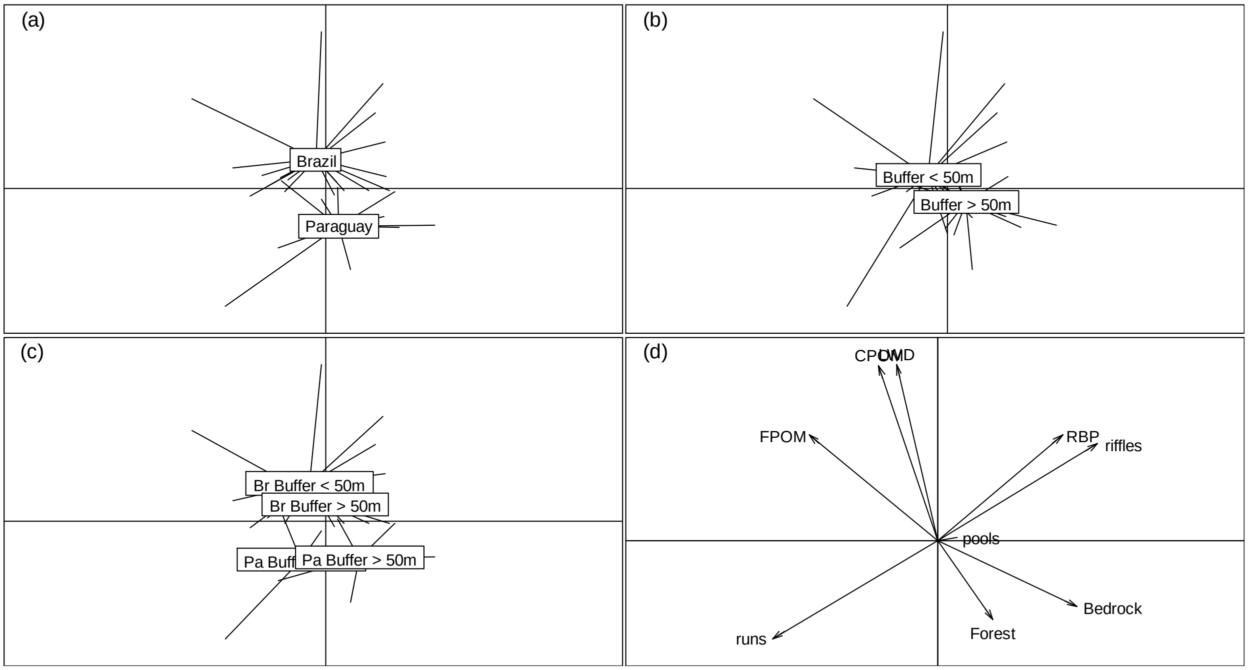

Figure 3. PCA ordination of habitat quality parameters for sampling sites in the two study regions (Brazil and Paraguay) (a), the high and low buffer width groups (b), and the high and low buffer width groups within each region (c). For Paraguay sites that were sampled twice, parameter values were averaged.

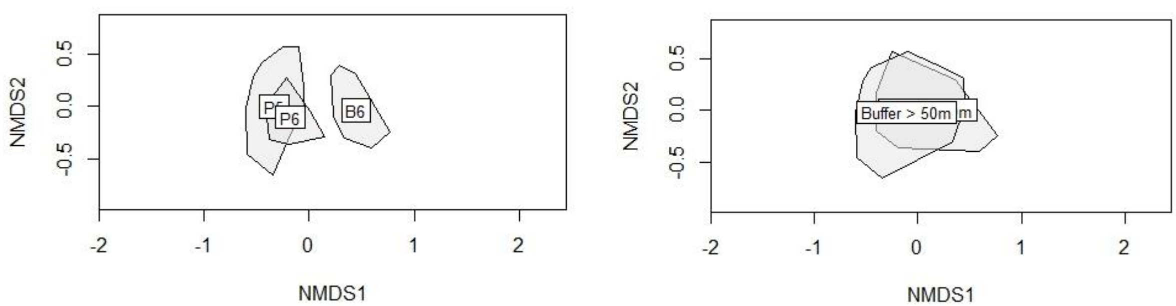

Figure 4. NMDS results showing sample groups for two events in Paraguay (P5 and P6) and one event in Brazil (B6) and sample groups for sites with low buffer width (<50m) and high buffer width (>50m).

Table 1. Summary statistics of site characteristics in each region

Parameter	Paraguay[a]	Brazil
Maximum depth (m)	0.50 ± 0.19	0.29 ± 0.16
Maximum width (m)	5.6 ± 1.5	3.8 ± 1.4
Maximum velocity (m/s)	0.45 ± 0.18	0.55 ± 0.15
Gradient (%)	2.4 ± 1.4	4.5 ± 3.5
Elevation (m)	232 ± 81	511 ± 73
Catchment size (Ha)	1604 ± 1226	924 ± 1134
% Cultivated [b]	70.96 ± 11.55	87.66 ± 5.27
Minimum buffer width (m)	89.3 ± 93.4	56.6 ± 87.3
RBP score	155 ± 25	162 ± 12
Water temperature (C)	20.7 ± 1.4	20.6 ± 1.0
Conductivity (uS/cm)	68 ± 15	32.4 ± 12.9
Dissolved oxygen (mg/L)	8.37 ± 1.0	8.79 ±1.3
Water turbidity	14.8 ± 6.6	17.1 ± 7.6
% sediment fines (silt and clay)	39.72 ± 14.59	65.9 ± 11.6
% sediment TOC	0.78 ± 0.46	2.32 ± 0.68
Total insecticide TU	0.29 ± 0.24	0.21 ± 0.24
Pyrethroid TU	0.23 ± 0.24	0.20 ± 0.23
% soft depositional sediment	28.5 ± 34.8	21.4 ± 18.5
% bedrock	37.1 ± 34.3	16.7 ± 18.1
% large woody debris	5.6 ± 4.2	9.8 ± 6.6
% fine particulate organic matter	2.4 ± 1.1	6.8 ± 4.3
% riffles	29.6 ± 16.5	39.2 ± 25.6
% pool	5.4 ± 7.5	3.6 ± 4.1

Table 2. Detection frequencies and maximum toxic units (TUs) for each sampling event, for insecticides that had at least one TU value >0.01. TUs were calculated as the ratio of the carbon-normalized concentration in sediment over the carbon-normalized LC50. Insecticide concentrations were reported in Hunt et al. (2016).

Pesticide	LC50 (ng/g organic carbon)	Statistic	Paraguay Jan 2013	Paraguay Dec 2013	Brazil Nov 2013
Chlorpyrifos	4160[a]	Max TU	0.15	0.05	0.02
		Frequency[b]	56%	77%	83%
Endosulfan	960[c]	Max TU	0.01	0.04	0.02
		Frequency[b]	13%	0%	0%
End. sulfate	5220[c]	Max TU	0.05	0.01	0.01
		Frequency[b]	6%	8%	0%
Cypermethrin	380[a]	Max TU	0.19	0.27	0.83
		Frequency[b]	31%	8%	44%
L-cyhalothrin	450[a]	Max TU	1.77	0.61	0.16
		Frequency[b]	6%	8%	39%
Bifenthrin	520[a]	Max TU	0.00	0.14	0.13
		Frequency[d]	38%	31%	44%
Cyfluthrin	1080[a]	Max TU	<QL	0.05	<QL
		Frequency[d]	13%	38%	11%
Deltamethrin	790[a]	Max TU	<QL	nd	0.06
		Frequency[d]	13%	0%	6%
Esfenvalerate	1540[a]	Max TU	<QL	nd	0.01
		Frequency[d]	38%	0%	22%
Permethrin	10830[a]	Max TU	0.02	0.01	0.01
		Frequency[d]	13%	0%	33%
Total pyrethroid TU [e,g]		Max TU	1.85	0.77	1.03
Total insecticide TU [f,g]		Max TU	1.89	0.84	1.07

[a] LC50 for Hyalella azteca from Weston et al. 2013

[b] Frequency of detection above the highest quantitation limit of 0.5 ng/g dw in sediment.

[c] LC50 for Chironomus tentans from You et al. 2005

[d] Frequency of detection above the highest quantitation limit of 0.25 ng/g dw in sediment

[e] Total pyrethroid TU values for each sample were calculated by summing the TU values for each pyrethroid.

[f] Total insecticide TU values for each sample were calculated by summing the TU values for each insecticide.

[g] A concentration value of half the quantitation limit was assigned for pesticides detected in the sample group but not detected in the sample, or detected < QL in the sample.

Table 3. Selected model results and relative importance of predictor variables included in averaged models. Includes only response metrics for which significant correlations with predictor variables were found ($p < 0.05$), and models selected for averaging were those with and $\Delta AIC<4$. The coefficient sign for each parameter is indicated after the relative importance value in brackets as (+) for positive correlation and (-) for negative correlation. Parameters that are significantly correlated with response value (average p-value of selected models < 0.05) are indicated with an asterisk. Parameters that are both significantly correlated and have moderated or high importance (>0.4) have values in bold.

Region	Response Metric	Selected Models		Relative Variable Importance												
		Adj. r2	AICc	% Agriculture	Buffer width	Insecticide TU	RBP	% Riffles	Turbidity	Soft sediment	CPOM	FPOM	LWD	TOC	Catchment area	Gradient
Brazil	%EPT	0.34 to 0.79	147.8 to 151.7	**0.62 (-)***	0.10 (+)		0.66 (-)	0.12 (-)*	0.17 (+)*	**0.55 (-)***	**0.40 (+)***	0.22 (-)	0.03 (-)	0.29 (-)	0.58(+)	
	%EPT-HB	0.51 to 0.63	125.3 to 129.0	0.07 (-)	**0.51 (+)**			**0.62 (-)***		**0.71 (-)***	0.06(+)				**1.0 (+)***	
	% Ephem.	0.25 to 0.60	141.7 to 145.7	**0.75 (-)***	**0.59 (+)***	0.03(-)	0.45(-)	0.04(-)		0.19(-)	0.32(+)	0.04(-)	0.10 (-)*		**0.93 (+)***	
	% Trich.	0.17 to 0.45	98.1 to 102.0	0.20 (-)	0.07 (-)	0.05 (+)	0.05(-)	0.14(-)		0.03(-)	**0.83 (+)***	**0.83 (-)***	0.04(-)	0.03(+)	0.14(-)	0.03(-)
	Coleop. richness	0.18 to 0.52	41.1 to 45.0	0.03 (-)	0.14 (+)	0.19 (-)	0.03 (+)		0.38 (-)	**0.80 (-)***		**0.80 (+)***		0.18 (+)	0.39 (-)	
Paraguay	%EPT	0.78 to 0.88	119.5 to 121.8	**0.76 (-)***			**1.0 (+)***	**1.0 (-)***					**1.0 (-)***	**1.0 (+)***	**1.0 (+)***	
	%EPT-HB	0.27 to 0.50	119.5 to 123.5			0.39 (+)	0.07 (+)*	0.07 (-)*	0.42 (+)	**0.93 (-)***	0.06 (+)		0.07 (-)*	0.43 (-)	0.17 (+)	
	% Ephem.	0.19 to 0.71	116.6 to 120.6			**0.86 (+)***	0.67 (+)	0.34 (-)		0.21 (-)	0.19 (+)		0.09 (-)	0.15 (-)	0.20 (+)	0.30 (-)
	% Trich.	0.19 to 0.49	111.0 to 114.9		0.02 (-)	0.18 (-)	0.14 (+)	0.46 (+)	**0.72 (+)***	0.28 (-)					0.31 (+)	
		Adj. r2	AICc	% Agriculture	Buffer width	Insecticide TU	RBP	% Riffles	Turbidity	Soft sediment	CPOM	FPOM	LWD	TOC	Catchment area	Gradient

EPT richness	0.28 to 0.54	56.1 to 59.7			0.66 (+)	**0.89 (+)***	0.03 (-)		0.16 (-)	0.04 (-)	0.32 (-)	0.30 (-)			
Trich. Richness			0.02 (+)	0.05 (-)	0.44 (+)	**0.83 (+)***	0.06 (+)*	0.06 (+)	0.16 (-)	0.06 (+)	**0.76 (-)***	0.07 (+)	0.14 (+)		0.02 (-)
Rare & Sensitive	0.40 to 0.74	47.0 to 51.0				**1.0 (+)***		**0.63 (-)***	**1.0 (+)***			**1.0 (-)***	0.38 (-)*		**1.0 (-)***
Shannon diversity	0.25 to 0.42	9.1 to 13.1				**0.81 (+)***		0.06 (-)	0.19 (-)*	0.06 (-)		0.10 (-)			**0.64 (-)***
Dominant taxon	0.27 to 0.47	124.0 to 127.9				**1.0 (-)***			0.10 (-)			0.09 (+)	0.08 (+)	0.16 (-)	**0.77 (+)***
3 dominant taxa	0.28 to 0.43	119.0 to 123.6	0.04 (+)	0.04 (-)	0.05 (-)	0.23 (-)	0.05 (-)	0.04 (+)	**0.86 (+)***	0.04 (+)	0.04 (-)	0.05 (+)	0.04 (+)	0.04 (-)	0.12 (+)
BMWP	0.34 to 0.69	141.3 to 145.2		0.02 (-)	0.05 (+)	**1.0 (+)***	0.13 (-)	0.51 (-)	0.16 (+)	0.48 (+)	0.22 (+)	0.02 (+)		0.03 (-)	0.07 (-)*
ASPT	0.21 to 0.73	10.9 to 14.9	0.46 (-)	0.16 (+)	**0.83 (+)***	0.62 (+)	0.12 (-)*		0.05 (-)	0.08 (+)*	0.06 (-)	0.19 (-)	0.13 (-)	0.01 (-)	0.27 (+)
SPEAR	0.56 to 0.75	92.0 to 96.0	0.14 (+)	0.13 (-)		**0.71 (+)***	0.03 (+)	**0.97 (+)***	**0.41 (-)***		0.05 (+)	0.58 (-)	0.02 (-)	0.07 (+)	0.04 (-)

Table S1. Sampling Sites and Schedule

Site Name	Latitude	Longitude	Jan -13	Nov-13	Dec -13
BR-02	24 48 44.4S	53 42 31.6W		X	
BR-03	24 48 51.4S	53 38 22.2W		X	
BR-07	24 57 31.2S	53 40 53.5W		X	
BR-10	24 56 54.4S	53 41 52.5W		X	
BR-11	24 56 31.8S	53 42 49.1W		X	
BR-12	24 45 49.1S	53 48 55.7W		X	
BR-13	24 44 41.6S	53 51 40.3W		X	
BR-14	24 44 40.8S	53 51 59.3W		X	
BR-15	24 45 28.2S	53 52 55.8W		X	
BR-16	24 45 05.2S	53 53 03.6W		X	
BR-17	24 44 57.4S	53 54 18.2W		X	
BR-18	24 47 43.0S	53 54 11.0W		X	
BR-19	24 49 19.8S	53 54 12.2W		X	
BR-20	24 49 07.5S	53 50 29.2W		X	
BR-21	24 48 29.6S	53 45 44.8W		X	
BR-22	24 45 48.2S	53 37 54.1W		X	
BR-23	24 47 48.0S	53 36 16.8W		X	
BR-24	24 47 57.2S	53 36 01.7W		X	
SD 01	26 42 21.2S	55 31 49.1 W	X		X
SD 02	26 45 48.7S	55 33 34.7W	X		
SD 03	26 49 34.5S	55 31 36.8W	X		
SD 04	26 53 29.8S	55 34 19.1W	X		X
SD 05	26 55 54.1S	55 31 00.8W	X		X
SD 06	26 52 12.5S	55 29 49.7W	X		X
SD 07	26 37 45.8S	55 39 55.1W	X		X
SD 08	26 47 37.0S	55 37 33.4W	X		X
SD 09	26 47 59.4S	55 35 38.0W	X		X
SD 10	26 50 08.3S	55 36 33.2W	X		X
SD 11	26 50 35.0S	55 36 43.7W	X		
SD 12	26 31 29.1S	55 37 06.7W	X		
SD 13	26 32 18.4S	55 37 0.8W	X		X
SD 14	26 26 19.0S	55 34 32.4W	X		X
SD 15	26 30 56.5S	55 36 43.3W			X
SD 16	26 28 21.9S	55 34 07.9W	X		X
SD 17	26 28 27.90S	55 33 57.40W			X

Table S2. Summary statistics of site characteristics in each region

Parameter	Statistic	Paraguay[a]	Brazil
Maximum depth (m)	minimum	0.27	0.12
	maximum	0.81	0.70
	median	0.44	0.25
	mean	0.50	0.29
	standard deviation	0.19	0.16
Maximum width (m)	minimum	3.8	2.0
	maximum	8.5	8.0
	median	5.5	3.3
	mean	5.6	3.8
	standard deviation	1.5	1.4
Maximum velocity (m/s)	minimum	0.18	0.24
	maximum	0.80	0.85
	median	0.46	0.55
	mean	0.45	0.55
	standard deviation	0.18	0.15
Gradient (%)	minimum	0.9	0.8
	maximum	5.0	11.1
	median	2.0	3.1
	mean	2.4	4.5
	standard deviation	1.4	3.5
Elevation (m)	minimum	130	400
	maximum	360	600
	median	200	485
	mean	232	511
	standard deviation	81	73
Catchment size (Ha)	minimum	224	75
	maximum	3591	5042
	median	1254	686
	mean	1604	924
	standard deviation	1226	1134
% Cultivated [b]	minimum	31.60	74.35
	maximum	82.30	93.37
	median	73.60	89.38
	mean	70.96	87.66
	standard deviation	11.55	5.27
Minimum buffer width (m)	minimum	3.0	9.0
	maximum	500.0	350.0
	median	100.0	32.5
	mean	89.3	56.6
	standard deviation	93.4	87.3

RBP score	minimum	79	134
	maximum	182	180
	median	162	164
	mean	155	162
	standard deviation	25	12
Water temperature (C)	minimum	17.6	19.0
	maximum	24.0	22.8
	median	21.0	20.7
	mean	20.7	20.6
	standard deviation	1.4	1.0
Conductivity (uS/cm)	minimum	34	14
	maximum	91	60
	median	70	30.5
	mean	68	32.4
	standard deviation	15	12.9
Dissolved oxygen (mg/L)	minimum	2.90	7.30
	maximum	10.00	14.60
	median	8.42	8.50
	mean	8.37	8.79
	standard deviation	1.0	1.3
Water turbidity	minimum	8.3	3.23
	maximum	28.4	31
	median	11.8	16.15
	mean	14.8	17.1
	standard deviation	6.6	7.6
% sediment fines (silt and clay)	minimum	14.00	44.6
	maximum	65.13	83.1
	median	37.10	65.7
	mean	39.72	65.9
	standard deviation	14.59	11.6
% sediment TOC	minimum	0.22	1.37
	maximum	2.12	3.24
	median	0.69	2.39
	mean	0.78	2.32
	standard deviation	0.46	0.68
Total insecticide TU	minimum	0.07	0.03
	maximum	1.01	1.07
	median	0.20	0.14
	mean	0.29	0.21
	standard deviation	0.24	0.24
Pyrethroid TU	minimum	0.04	0.03
	maximum	0.98	1.03

	median	0.15	0.13
	mean	0.23	0.20
	standard deviation	0.24	0.23
% soft depositional sediment	minimum	0.0	0
	maximum	100.0	70
	median	7.5	17.5
	mean	28.5	21.4
	standard deviation	34.8	18.5
% bedrock	minimum	0.0	0
	maximum	95.0	50
	median	35.0	10
	mean	37.1	16.7
	standard deviation	34.3	18.1
% large woody debris	minimum	0.0	2
	maximum	15.0	25
	median	5.0	7.5
	mean	5.6	9.8
	standard deviation	4.2	6.6
% fine particulate organic matter	minimum	2.0	2
	maximum	5.0	20
	median	2.0	5
	mean	2.4	6.8
	standard deviation	1.1	4.3
% riffles	minimum	0.0	5
	maximum	60.0	90
	median	30.0	35
	mean	29.6	39.2
	standard deviation	16.5	25.6
% pool	minimum	0.0	0
	maximum	20.0	10
	median	0.0	2.5
	mean	5.4	3.6
	standard deviation	7.5	4.1

[a]For parameters that were measured during both sampling events in Paraguay, statistics are based on values that were averaged over both sampling events.

[b]Cultivated area was based on non-forested area, estimated with LANDSAT data.

Table S3. Invertebrate community response metrics evaluated.

Response Metric	Description
% Ephemeroptera	Relative abundance of Ephemeroptera (all families)
% Plecoptera	Relative abundance of plecoptera (all families)
% Trichoptera	Relative abundance of Trichoptera (all families)
% EPT	Relative abundance of Ephemeroptera, Plecoptera, and Trichoptera (all families)
% EPT-HB	Relative abundance of Ephemeroptera (all families except Baetidae), Plecoptera (all families), and Trichoptera (all families except Hydropsychidae)
% Chironomidae	Relative abundance of Chironomidae
% Diptera	Relative abundance of Diptera (all families)
% Elmidae	Relative abundance of Elmidae
% Coleoptera	Relative abundance of Coleoptera (all families)
% Oligochaeta	Relative abundance of Oligochaeta (all families)
% Gastropoda	Relative abundance of Gastropoda (all families)
% Bivalvia	Relative abundance of Bivalvia (all families)
% Non insects	Relative abundance of all non-insect taxa
% Crustacea	Relative abundance of Crustacea (all families)
EPT richness	Richness of Ephemeroptera, Plecoptera, and Trichoptera families
Trichoptera richness	Richness of Trichoptera families
Coleoptera richness	Richness of Coleoptera families
Density	Total abundance of all taxa per m^2
$SPEAR_{pesticides}AR$	Species at Risk pesticides index modified for Argentina (Chapter 4)
$SPEAR_{pesticide}EU$	Species at Risk pesticides index - European version
RareSens	Rare and sensitive taxa presence (0 to 5), including Corydalidae, Grypopterygidae, Perlidae, Aeglidae, and Trichodactylidae
BMWP	Biological Monitoring Working Party score modified for Atlantic Forests
ASPT	BMWP average score per taxon score modified for Atlantic Forests
Total richness	Total taxa richness, rarefied to sample size of 300
Shannon-Weaver	Shannon-Weaver diversity index
% Dom1	Relative abundance of most dominant taxon
% Dom3	Relative abundance of three most dominant taxa

Table S4 Relative abundance and detection frequency of macroinvertebrate taxa and the Paraguay and Brazil study streams

	Paraguay		Brazil	
	Relative Abundance (%)	% Samples detected	Relative Abundance (%)	% Samples detected
Coleoptera				
Chrysomelidae	0.007	2.9	0.000	0.0
Dryopidae	0.026	2.9	0.023	11.1
Dytiscidae	0.013	2.9	0.023	11.1
Elmidae	21.200	97.1	12.457	100.0
Gyrinidae	0.007	2.9	0.011	5.6
Hydrophilidae	0.007	2.9	0.000	0.0
Lutrochidae	0.026	11.4	0.080	22.2
Psephenidae	0.765	57.1	0.616	50.0
Scarabaeidae	0.007	2.9	0.000	0.0
Diptera				
Ceratopogonidae	0.310	48.6	0.856	83.3
Chironomidae	28.619	100.0	49.452	100.0
Empididae	0.053	17.1	2.407	100.0
Muscidae	0.007	2.9	0.034	11.1
Psychodidae	0.132	28.6	0.080	16.7
Simuliidae	1.576	65.7	3.034	94.4
Tabanidae	0.007	2.9	0.023	11.1
Tipulidae	0.264	34.3	0.422	66.7
Ephemeroptera				
Baetidae	7.122	100.0	6.251	88.9
Caenidae	0.653	54.3	0.046	16.7
Leptohyphidae	12.443	100.0	0.068	22.2
Leptophlebiidae	1.879	88.6	7.506	88.9
Hemiptera				
Belostomatidae	0.000	0.0	0.068	5.6
Corixidae	0.007	2.9	0.000	0.0
Gerridae	0.013	5.7	0.023	11.1
Helotrephidae	0.317	14.3	0.011	5.6
Mesoveliidae	0.007	2.9	0.000	0.0
Naucoridae	0.930	60.0	0.034	11.1
Pleidae	0.178	8.6	0.000	0.0
Veliidae	0.092	11.4	0.411	55.6
Lepidoptera				
Crambidae	0.270	37.1	0.000	0.0
Megaloptera				
Corydalidae	0.244	31.4	0.137	27.8
Odonata				
Calopterygidae	0.185	8.6	0.068	27.8

Coenagrionidae	0.409	31.4	0.000	0.0
Gomphidae	0.580	65.7	0.274	55.6
Megapodagrionidae	0.013	5.7	0.011	5.6
Libellulidae	0.369	60.0	0.103	33.3
Protoneuridae	0.007	2.9	0.000	0.0
Plecoptera				
Grypopterygidae	0.053	11.4	0.148	16.7
Perlidae	0.204	28.6	0.422	50.0
Trichoptera				
Calamoceratidae	0.099	28.6	0.970	50.0
Hydroptilidae	0.626	62.9	0.354	55.6
Helicopsychidae	0.020	2.9	0.000	0.0
Hydrobiosidae	0.092	14.3	0.034	16.7
Hydropsychidae	6.686	88.6	3.822	100.0
Glossosomatidae	1.701	82.9	0.091	22.2
Leptoceridae	0.244	42.9	0.137	33.3
Limnephilidae	0.000	0.0	0.034	5.6
Odontoceridae	1.002	42.9	0.011	5.6
Philopotamidae	0.818	62.9	0.399	22.2
Polycentropodidae	0.336	17.1	0.000	0.0
Sericostomatidae	2.123	42.9	0.000	0.0
Collembola				
Entomobryonidae	0.046	8.6	0.000	0.0
Acari	0.534	57.1	0.068	16.7
Bivalvia	0.659	40.0	4.472	66.7
Gastropoda	2.466	68.6	0.160	44.4
Turbellaria	0.468	42.9	0.046	16.7
Hirudinea	0.079	22.9	0.046	16.7
Nematoda	0.185	45.7	0.000	0.0
Nemertea	0.033	14.3	0.000	0.0
Oligochaeta	1.774	88.6	2.350	100.0
Aeglidae	0.000	0.0	1.666	77.8
Trichodactylidae	0.119	34.3	0.000	0.0
Ostracoda	0.000	0.0	0.023	11.1
Amphipoda	0.020	8.6	0.091	11.1

CHAPTER 6

Conclusions and future directions

Conclusions and future directions

The results of my dissertation research demonstrated that: (1) there was consistency in the insecticides that were most commonly detected in sediment samples from streams in the intensive soy production regions studied in Argentina, Brazil and Paraguay; (2) these insecticides, especially the pyrethroids, persisted in stream sediments at concentrations likely to cause acute and chronic toxicity to aquatic invertebrates; and, (3) acutely toxic insecticide concentrations in sediments were most likely to occur in streams with buffer widths less than 20m.

With respect to the impact of insecticides on stream invertebrate communities, results differed between the Pampas study regions in Argentina and the Atlantic Forest regions in Brazil and Paraguay. Chapter 4 demonstrated a correlation between insecticide toxic levels in stream sediments and changes in aquatic invertebrate communities of the Argentine Pampas. The $SPEAR_{pesticides}$ index consistently showed a significant decrease with increasing insecticide TUs, and insecticide TU was the most important variable in explaining variability in the $SPEAR_{pesticides}$ index, indicating that it is relatively insensitive to non-pesticide stressors. However, Chapter 5 showed that in the Paraguay and Brazil study regions there was no clear correlation between insecticide toxic levels and $SPEAR_{pesticides}$ or other invertebrate community metrics. Results indicated that non-pesticide agricultural stressors were more important than insecticides in affecting invertebrate communities. In particular, the amount of soft depositional sediment had high importance in explaining variability in several invertebrate community metrics in both Paraguay and Brazil, and the RBP habitat score was very important in explaining variability in multiple metrics in Paraguay.

Crustaceans, especially amphipods in the genus *Hyalella*, comprised a large part of the stream invertebrate communities in the Argentine Pampas, and also played an important role as sensitive taxa in performance of the $SPEAR_{pesticides}$ index in that region.Chapter 3 showed that the most common native species of *Hyalella*is highly sensitive to the four insecticides most commonly detected.Interestingly, amphipods were rare in Atlantic forest streams in Paraguay and Brazil, likely because the habitat was unsuitable. The absence of these sensitive crustaceans may help explain why the $SPEAR_{pesticides}$ index did not perform well in streams of the Atlantic forest regions. While some taxa in Atlantic forest streams may have similar sensitivity to pesticides as amphipods, these other taxaare more likely to also be sensitive to many habitat and water quality variables in addition to pesticides, potentially confounding the analysis. Amphipods are very sensitive to pesticides but relatively tolerant of many habitat and water quality parameters (Chapters 3 and 4). In contrast, other sensitive and abundant taxa (such as EPT taxa) tend to be sensitive not only to pesticides but also to many habitat and water quality parameters (Bunzel et al., 2013).When the SPEAR index threshold values were optimized for Argentine Pampas streams, only the crustaceans and trichopterans were considered sensitive to pesticides, and amphipods were the most abundant sensitive organisms, making them very important in the performance of the SPEAR index. The role of *Hyalella* amphipods in Argentine streams contrasts with the role of *Gammarus*amphipods in the $SPEAR_{pesticides}$ index in Europe. Although *G. pulex* has high physiological sensitivity to pesticides, this species can migrate very fast and thus is considered not at risk to pesticides(Liess and Von der Ohe, 2005). I was unable to find data on the migration rate of *Hyalella* species, but the decrease in relative abundance of amphipods corresponding with increase in insecticide toxic units demonstrates that *Hyalella* should be considered to have high overall sensitivity to insecticides.

The presence of forested riparian buffers along the entire lengths of all streams may also help explain why $SPEAR_{pesticides}$ index values were not significantly correlated with insecticide TU values in either of the Atlantic forest regions. The forested riparian zones are likely to increase the resilience and recovery ability of invertebrate communities. There is ample evidence that forested headwaters provide reservoirs of invertebrate populations that assist in the recovery of downstream communities after disturbance (Liess and Ohe, 2005; Orlinskiy et al., 2015). In costrast, the stream buffers in Argentina were much smaller and were not forested, thus the invertebrate communities may not have been as resilient.

It is also possible that the relative effects of buffer widths in protecting invertebrate communities were less evident in my Atlantic Forest study than in other similar studies in which streams generally had much smaller buffer width. Although there were some differences in relative buffer width, all of the streams in both of my Atlantic Forest study regions can be considered well protected in comparison to streams in many other intensive agricultural regions throughout the world. In contrast, previous studies that have found riparian buffer zones to be effective in mitigating effects on stream invertebrate communities have generally evaluated streams with much smaller protected buffer zones than those considered in my study (Whiles et al. 2000;Rios and Bailey, 2006). In my Brazil study area, where buffer widths were generally lower than those in Paraguay, buffer width did have moderate importance in explaining variability in several invertebrate metrics, while in Paraguay it had little or no importance.Although results indicated that riparian buffer width was a moderately important predictor variable in Brazil, but had low importance in Paraguay, it is likely that the findings in Paraguay were limited by the lack of variation in minimum buffer width in that region. Approximately half of the Paraguay sites had minimum buffer widths of 100m, which was the minimum required by law.

My study results corroborate findings from other studies that have found riparian buffer zones to be important in mitigating transport of pesticides to streams. The present study’s finding of the highest TU values in streams with buffer widths less than 20 m was within the range of buffer widths (5 m to 20 m) reported to mitigate pesticide effects on streams (Rasmussen et al. 2011; Di Marzio 2010; Bunzel et al. 2014; Reichenberger et al. 2007). Many factors could affect the buffer width necessary to protect streams from pesticide exposure, including gradient, type of vegetation, soil properties, types of pesticides applied, timing and amount of pesticides applied, and presence of tile drains or drainage ditches that short-circuit the buffer zones (Reichenberger et al. 2007; Bunzel et al. 2014). Although regulation of pesticide mitigation measures often focuses on application practices, landscape level mitigation measures, such as requiring riparian buffer zones, may be easier to implement and enforce. For example, Bereswill et al. (2014) reviewed the efficacy and practicality of risk mitigation measures for diffuse pesticide entry into aquatic ecosystems, and ranked riparian buffer strips as highly effective for mitigating both spray drift and runoff, with high acceptability and feasibility. Riparian buffer zones are required to be maintained in both Brazil and Paraguay, although specific requirements are in flux. However, the implementation and enforcement of new riparian buffer requirements in Brazil has been difficult and controversial, especially in regions with small-scale production where a significant amount of a landowner’s productive farmland could be lost with compliance (Alvez et al. 2012). More research is needed to determine the effectiveness of current and proposed riparian buffer regulations in Paraguay and Brazil in protecting stream habitat, water quality, and ecosystems.

To my knowledge, the results I present in Chapter 4 constitute the first field study that has demonstrated such effects of insecticides on invertebrate communities in soy production regions.

In intensive soy production regionsinthe midwest region of the United States,as well as in Brazil and Argentina, many studies have reported frequent detections of insecticides, as well as toxicity to specific sensitive species (Casara et al., 2012; Di Marzio et al., 2010; Ding et al., 2010; Hladik and Kuivila, 2012; Jergentz et al., 2004a, 2004b; Laabs et al., 2002; Mugni et al., 2011). However, none of these studies investigated effects on entire invertebrate communities over a gradient of pesticide concentrations.

This research was the first application of the SPEAR index in South America, and the first to use it to evaluate effects of pesticides on invertebrate communities associated with aquatic vegetation. The $SPEAR_{pesticides}$ index performed equally well for aquatic invertebrate communities associated with emergent vegetation as it did for benthic invertebrate communities. The results of my research are consistent with previous studies that have shown the $SPEAR_{pesticides}$ index to not be highly influenced by non-pesticide variables (Beketov and Liess, 2008; Liess et al., 2008a, 2008b). Although the SPEAR index was developed in Europe, it performed well in the Argentine Pampas with only minor modifications, and would likely improve as more data are obtained on South American taxa traits such as generation time and migration rates.

This research did not find a correlation between the $SPEAR_{pesticides}$ index and insecticide TU values in the Atlantic forest streams included in the study. However, the fact that almost all streams had ample forested riparian buffer zones is likely to have mitigated the effects of pesticides on stream invertebrate communities. The $SPEAR_{pesticides}$ index may still prove to be a useful tool in Atlantic Forest streams without much of a riparian buffer, especially if wider ranges of pesticide concentrations occur.

Chapters 3 and 4 contribute valuable information on pesticide sensitivity of an important South American amphipod species with laboratory studies as well as field studies, and indicates that its sensitivity is very similar to the closely related North America species *H. azteca*. A repeated criticism is the lack of sensitivity data on species that occur outside of Europe and North America, and there may be reason to believe that sensitivities could be different in species occurring in the Southern hemisphere and near the equator. For example, Kwok et al. (2007) found that tropical species may be more sensitive than temperate species to pesticides, while temperate species are likely to be more sensitive to metals. More studies are needed on a range of organisms to determine whether use of sensitivity data for northern hemisphere species are adequately protective.

Although the neonicotinoid insecticides were not analyzed as part of the present study because there was little evidence of their use at the start of field work, it is likely that their use in the soy production in South America has increased in recent years, and will continue to increase. In South America, neonicotinoids are often applied in combination with pyrethroids for control of hemipteran pests in soy. In Argentina, there are at least 57 neonicotinoid/pyrethroid mixture formulations registered for this purpose, although not all of them are currently in commercial use (Servicio Nacional de Sanidad y Calidad Agroalimentaria, personal communication, Dec 2013). Recent studies in soy production regions of South America detected imidacloprid in 43% of surface water samples (Argentina; de Geronimo et al. 2014) and thiamethoxam in 100% of surface water samples (Brazil; Rocha et al. 2015). A review of neonicotinoid studies around the world found that 81% of the studies found maximum surface water concentrations that exceeded an acute toxicity threshold, and 74% found average concentrations that exceeded a chronic threshold (Morrissey et al. 2014). Future studies in soy production regions of South America should include analysis of the occurrence and effects of neonicotinoids in aquatic ecosystems.

My results suggest that the following recommendations should be considered in soy production regions of South America: (1) evaluation and implementation of buffer zones and other management practices to limit transport of pesticides to streams; (2) field studies focusing on effects to aquatic invertebrate communities; and, (3) continued monitoring that is adapted based on quickly changing pesticide use trends.

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