Contents lists available at ScienceDirect



# Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



# High sensitivity of invertebrate detritivores from tropical streams to different pesticides

Aydeé Cornejo<sup>a,\*,1</sup>, Francisco Encina-Montoya<sup>b</sup>, Francisco Correa-Araneda<sup>c</sup>, Dalys Rovira<sup>d</sup>, Gabriela García<sup>a</sup>, Carlos Nieto<sup>a</sup>, Víctor Villarreal<sup>d</sup>, Nicomedes Jaramillo<sup>e</sup>, Edgar Pérez<sup>a</sup>, Anayansi Valderrama<sup>a</sup>, Javier Pérez<sup>f</sup>, Luz Boyero<sup>f,g</sup>

<sup>a</sup> Aquatic Ecology and Ecotoxicology Laboratory, Zoological Collection Eustorgio Mendez, Gorgas Memorial Institute of Health Studies, (COZEM-ICGES), Ave. Justo Arosemena and Calle 35, 0816-02593 Panama City, Panama

<sup>b</sup> Núcleo de Estudios Ambientales, Departamento de Ciencias Ambientales, Facultad de Recursos Naturales, Universidad Católica de Temuco, 4780000 Temuco, Chile

<sup>c</sup> Unidad de Cambio Climático y Medio Ambiente (UCCMA), Instituto Iberoamericano de Desarrollo Sostenible (IIDS), Universidad Autónoma de Chile, Temuco, Chile <sup>d</sup> Water Laboratory and Physicochemical Services (LASEF), Autonomous University of Chiriqui, David City, Panama

<sup>e</sup> Research Center for Natural Products and Biotechnology (CIPNABIOT), Autonomous University of Chiriqui, David City, Panama

<sup>f</sup> Department of Plant Biology and Ecology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain

<sup>g</sup> IKERBASQUE, Bilbao, Spain

# ARTICLE INFO

Edited by: Dr Yong Liang

Keywords: Agriculture Detritivores Freshwater Fungicides Insecticides Toxicity bioassays The tropics

#### ABSTRACT

Freshwater organisms are often sensitive to pesticides, but their sensitivity varies across different taxa and with pesticide type and action mode, as shown by multiple acute toxicity tests. Such variability hampers predictions about how freshwater ecosystems may be altered by pesticide toxicity, which is especially critical for understudied areas of the world such as the tropics. Furthermore, there is little information about the sensitivity of some organisms that are key components of stream food webs; this is the case of litter-feeding detritivorous invertebrates, which contribute to the fundamental process of litter decomposition. Here, we examined the sensitivity of three common detritivores [Anchytarsus sp. (Coleoptera: Ptilodactylidae), Hyalella sp. (Amphipoda: Hyalellidae) and Lepidostoma sp. (Trichoptera: Lepidostomatidae)] to three pesticides commonly used (the insecticides bifenthrin and chlorpyrifos and the fungicide chlorothalonil) using acute (48 or 96 h) toxicity tests. Our study demonstrates that common-use pesticides provoke the mortality of half their populations at concentrations of 0.04–2.7  $\mu$ g L<sup>1</sup>. We found that all species were sensitive to the three pesticides, with the highest sensitivity found for chlorpyrifos. Additionally, we used the approach of species sensitivity distributions (SSD) to compare our study species with Daphnia magna and other temperate and tropical invertebrates. We found that the study species were among the most sensitive species to chlorpyrifos and chlorothalonil. Our results suggest that tropical detritivores merit special attention in ecological risk assessment of pesticides and highlight the need for accurate ecotoxicological information from ecologically relevant species in the tropics.

#### 1. Introduction

Tropical forests are declining at unprecedented rates in favor of agriculture (Gibbs et al., 2010), and such replacement causes severe impacts on stream ecosystems that are associated to multiple stressors (Rasmussen et al., 2016; Cornejo et al., 2019). Among these stressors, pesticides are of great importance as they are often toxic for freshwater organisms (Schäfer et al., 2011), causing sublethal and lethal effects and subsequent alterations in ecosystems (Bighiu et al., 2020; Bundschuh

et al., 2020; Cornejo et al., 2020b). Many studies have examined the toxicity of different pesticides on a variety of freshwater invertebrates through acute and chronic toxicity tests, but the great majority of these studies have been conducted with species from temperate areas, mostly Europe and North America (Rico et al., 2011). Because of this paucity of data, water quality criteria in most tropical regions rely on extrapolations of ecotoxicological data from temperate zones using standard temperate species, assuming that their sensitivities are similar (Kwok et al., 2007; Schäfer et al., 2013), even though the fate and effects of

\* Corresponding author.

https://doi.org/10.1016/j.ecoenv.2021.112226

Received 28 December 2020; Received in revised form 27 March 2021; Accepted 31 March 2021 Available online 10 April 2021 0147-6513/© 2021 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

E-mail address: acornejo@gorgas.gob.pa (A. Cornejo).

<sup>&</sup>lt;sup>1</sup> ORCID-ID: 0000-0001-6789-5847.

pesticides may be different between climatic regions (Daam and Rico, 2018). This is an important research gap that hinders accurate assessments of the environmental risk of pesticides in tropical streams.

Additionally, there is little information about how some groups of organisms are affected by pesticides, and this includes litter-feeding detritivorous invertebrates (hereafter detritivores), which are major components of stream food webs (Cummins et al., 1989), especially in forested headwaters (Vannote et al., 1980). Litter decomposition is a key process and a fundamental component of stream ecosystem functioning, mediated by microbial decomposers and detritivores (Graça, 2001). These invertebrates feed on litter and thus contribute to its fragmentation, the production of fine particulate organic matter that serves as food to collectors (Cummins and Klug, 1979), and nutrient recycling (López-Rojo et al., 2019). Even if detritivores are less abundant and species rich in the tropics than in temperate areas (Boyero et al., 2011), they still play an important ecological role (Boyero et al., 2012; Cheshire et al., 2005; Yule et al., 2010). For this reason, and because they often belong to sensitive taxa (e.g., Trichoptera, Amphipoda; Boyero et al., 2020), it becomes crucial to assess how detritivores are affected by the presence and concentration of pesticides in streams.

Here, we examined the sensitivity of three common tropical detritivores [Anchytarsus sp. (Coleoptera: Ptilodactylidae), Hyalella sp. (Amphipoda: Hyalellidae) and Lepidostoma sp. (Trichoptera: Lepidostomatidae)] to three pesticides commonly used in agricultural land in Central America and western Panama: the insecticides bifenthrin and chlorpyrifos and the fungicide chlorothalonil. We performed acute (48 or 96 h) toxicity tests to assess the mortality and to calculate the mean lethal concentration (LC<sub>50</sub>) of each pesticide for each species (with the only exception of the Lepidostoma – bifenthrin combination). We then used the approach of species sensitivity distributions (SSDs) to integrate acute toxicity data of multiple aquatic organisms from different trophic levels and geographic regions. SSDs are used to estimate hazardous concentrations (HC<sub>p</sub>) that affect p% of species (Posthuma et al., 2002). We predicted that (1) our study species would be sensitive to the three pesticides tested, despite the existence of (2) differences among species and (3) differences among pesticides, with the insecticides being more harmful than the fungicide; and (4) our study species would be more sensitive than temperate species, including the standard species D. magna.

# 2. Materials and methods

#### 2.1. Study area

Our study area was the upper catchment of the Chiriquí Viejo river, located on the Pacific coast of western Panama (8.25 - 9.00 °N, 82.25 -83.00 °W). Catchment area is 1376 km<sup>2</sup>, the length of the main river is 161 km and the highest altitude is 3474 m a.s.l. at the Barú Volcano (ETESA, 2008). The climate is tropical, with minimum, average and maximum air temperatures of 17.8, 28.0 and 35.5 °C, respectively (ANAM and CATIE, 2014). Total annual precipitation is 3400 mm on average, with a maximum of 7000 mm at high altitudes, 87.7% occurring in the wet season from May to December (ETESA, 2008). We conducted the study at the outdoor facilities of the Ministry of the Environment Station in the La Amistad International Park (PILA; 8.894 °N, 82.615 °W; MS Fig. S1), located ca. 400 m away from one of the streams where invertebrates were collected. This facilitated the collection and transport of invertebrates and water, and ensured similar temperature conditions between the collection sites and experimental location.

# 2.2. Pesticide selection

A total of 29 pesticides have been reported in the upper catchment of the Chiriquí Viejo river (Cornejo et al., 2019). We selected three for the present study based on their reported concentrations, persistence in the environment, physical properties, mechanisms of action and hazard (Table S1): the insecticides bifenthrin and chlorpyrifos and the fungicide chlorothalonil. Bifenthrin (C<sub>23</sub>, H<sub>22</sub> ClF<sub>3</sub>O<sub>2</sub>) is a pyrethroid insecticide commonly used in the control of foliage, which interferes with the so-dium channel; in our study area it has been reported in ranges from 0.15 to 0.35 µg L<sup>-1</sup>(Cornejo et al., 2019). Chlorpyrifos (C<sub>9</sub>H<sub>11</sub>Cl<sub>3</sub>NO<sub>3</sub>PS) is an organophosphate insecticide that acts as acetylcholinesterase (AChE) inhibitor it is widely used in agriculture and it has been reported in ranges from 0.14 to 15.24 µg L<sup>-1</sup> in our study area (Cornejo et al., 2019). Chlorothalonil (C<sub>8</sub>Cl<sub>4</sub>N<sub>2</sub>) is a polychlorinated aromatic compound that has been reported in ranges from 0.26 to 0.38 µg L<sup>-1</sup> in sediment in the study basin (MIDA, 2016), and it has been shown to be lethal for temperate detritivorous caddisflies in microcosms (Cornejo et al., 2020a).

The three pesticides were purchased at local agricultural stores authorized for the sale and distribution of agrochemicals. The ranges provided for the three pesticides are those reported in a previous study conducted in the same area (Cornejo et al., 2019; MIDA, 2016). The dilutions were made using filtered stream water from the Chiriquí Viejo river collected within the PILA, where no pesticides had been detected (Cornejo et al., 2020b). Two ranges were set for working solutions: a first range of low concentrations  $(0.01, 0.1, 0.5 \mu g L^{-1})$ , and a second range of high concentrations (1.0, 1.5, 2.5, 5.0 and 10.0  $\mu$ g L<sup>-1</sup>). The working solutions were prepared from two nominal or stock solutions: 100 mg L<sup>-1</sup> for the first range and 1000 mg L<sup>-1</sup> for the second range. Stock solutions were prepared based on the concentration of the active principle indicated in the commercial products, using micropipettes with plastic tips and glass volumetric flasks. Nominal concentrations were used because we could not make measurements during the experiment due to logistical and financial limitations. Thus, we acknowledge that our results can be compared with other studies but should not be used in regulatory risk assessment (Von Fumetti and Blaurock, 2018).

### 2.3. Taxon selection, sampling and acclimation

We obtained a list of common detritivores and their distribution in the Chiriquí Viejo river upper catchment from previous studies (Cornejo et al., 2019, 2020b), and chose three taxa that were dominant in the area: Anchytarsus sp., Hyalella sp. and Lepidostoma sp. We did not identify the species, but confirmed that all individuals belonged to the same species (although we name them as genera hereafter for simplicity). We collected these detritivores in 1st and 2nd order independent tributaries of the Chiriquí Viejo river located within protected areas to guarantee that individuals had not been previously exposed to pesticides. These areas where the Barú Volcano National Park (PNVB), where we collected specimens of Lepidostoma, and the PILA, where we collected Anchytarsus and Hyalella. We used two sampling techniques: litter bags filled with Alnus acuminata Kunth. (Betulaceae) that were submerged in the stream for 15 days, and multihabitat sampling using a 0.5 mm D-net. At each sampling site we used a multiparameter probe (HACH; HQ40d) to measure pH, temperature (°C), conductivity (µS cm<sup>-1</sup>), turbidity (NTU) and dissolved oxygen saturation (%); and a flowmeter (Flowatch 12, 300) to measure current velocity m s<sup>-1</sup> (Table S2).

The collected invertebrates were placed in plastic containers filled with stream water and litter and provided with constant aeration, and transferred to the experimental facilities where they were counted and identified using a stereoscope. They were then placed in aquariums containing 1–2 L of stream filtered water, where they were acclimated for 96 h with constant aeration (provided through syringe tips connected to a pump), at an air temperature of 17.1  $\pm$  0.8 °C, water temperature of 14.4  $\pm$  0.2 °C, and light: dark regime of 12:12 h (the natural conditions at the time of the experiment). They were fed with fragments of *A. acuminata* litter for the first 48 h of acclimation and then fasted for the last 24 h before starting the acute toxicity tests (Table S3).

# 2.4. Toxicity tests

The tests were conducted in 216 microcosms distributed in 67 treatments, which corresponded to different combinations of taxa (*Anchytarsus, Hyalella* and *Lepidostoma*), pesticide types (bifenthrin, chlorpyrifos and chlorothalonil) and pesticide concentrations [0 (control), 0.01, 0.1, 0.5, 1.0, 1.5, 2.5, 5.0 and 10.0  $\mu$ g L<sup>-1</sup>]; the only combinations that were absent, due to logistical constraints, were those with *Lepidostoma* and bifenthrin. Thus, there were 24 microcosms per combination of taxon and pesticide, and three replicates per concentration (except controls, N = 6–9; Table S4; Fig. S1). Each microcosm contained 200 mL of filtered (100  $\mu$ m) stream water, which was constantly aerated through syringe tips connected to an aquarium pump, and received five individuals of a given species (with 1080 individuals tested in total: 405 *Anchytarsus*, 405 *Hyalella* and 270 *Lepidostoma*).

Test methods were based on the Environmental Protection Agency standard protocol for toxicity testing with freshwater organisms (EPA, 2002). However, some environmental conditions were adapted to the ecological demands of the invertebrates tested. The experiment was performed in a static exposure regime with a single initial dose. During the experiment the air temperature was  $17.1 \pm 0.8$  °C, the water temperature was  $14.3 \pm 0.2$  °C and the light: dark regime was 12:12 h (which were the conditions in nearby streams at the time of the experiment). We assessed the effect of pesticide exposure using mortality as the end point. The tests lasted 48 (or 96 h in the case of *Anchytarsus* for bifenthrin and chlorothalonil) and lethal effects were monitored 24 and 48 h (or 96 h) after exposure, with dead organisms being removed.

# 2.5. Data analyses

Based on mortality at 48 or 96 h after exposure we calculated LC50 values (i.e., the concentration required to kill 50% of the population) and associated 95% confidence intervals (CIs) for each species and pesticide concentration, using the ecotox package [Hlina et al. (2019) in R statistical software (R Core Team, 2020)]. We used the approach of SSD and HC5 to compare our LC50 values with those reported for aquatic organisms of different functional groups [collectors (Co); detritivores (De); herbivores (Hb); omnivores (Om); primary producers (Pp) and predators (Pr)], separated by pesticides and geographic distribution (see summary of data used in Table S5-S7). For this, we used the data available at the ECOTOX database of the United States Environmental Protection Agency (USEPA; https://cfpub.epa.gov/ecotox/search.cfm). We selected data from the ECOTOX database following these criteria (partly as in Maltby et al., 2005): (1) endpoints were LC<sub>50</sub> or median effect concentration (EC<sub>50</sub>), regarding mortality or immobility for animals, and growth rate for algae; (2) test duration was 2-21 d for fish, and 1-7 d for invertebrates and algae; (3) the lowest value was selected when several duration values were studied in the same experiment; (4) the geometric mean was taken for data of the same species (and end point) but from different experiments; (5) data reported as  $\langle or \rangle$  were not used; (6) only static tests carried out in the laboratory were considered; and (7) only values published in scientific literature were used since 2000. We calculated the SSD using the ssdtools R package (Thorley and Schwarz, 2018), and built different SSDs for LC<sub>50</sub>/EC<sub>50</sub> for the three pesticides. The available distributions included the log-normal (lnorm), log-logistic (llog), and gamma distributions. The comparison between the sensitivity of tropical and temperate species to chlorpyrifos was verified using the Kolmogorov-Smirnov test.

# 3. Results

Mortality after 48 or 96 h of exposure was  $\leq 10\%$  in our control microcosms and corresponded to *Hyallela*. The results of the toxicity tests, LC<sub>50</sub> values (µg L<sup>1</sup>) and their 95% confidence intervals (CI) are shown in Table 1. LC<sub>50</sub> values indicated that chlorpyrifos was one order of magnitude more toxic than bifenthrin and chlorothalonil for the

#### Table 1

Results of the acute toxicity tests (24–96 h) with bifenthrin, chlorpyrifos and chlorothalonil for three detritivores, with mortality as endpoint; we show the  $LC_{50}$  and  $R^2$  of the test.

Pesticides	Species	Test duration (h)	LC <sub>50</sub> (95% CI; µg L <sup>-1</sup> )	R <sup>2</sup>
Bifenthrin	Anchytarsus	24	10.7 (6.63–35.9)	0.54
		48	2.86 (1.69-5.8)	0.63
		96	1.54 (0.74–3.0)	0.67
	Hyalella	24	4.30 (3.15–6.45)	0.49
		48	1.77 (1.28–2.4)	0.74
Chlorpyrifos	Anchytarsus	24	0.211 (0.106-0.345)	0.82
		48	0.0799 (0.0333-0.148)	0.83
	Hyalella	24	0.106 (0.04-0.22)	0.90
		48	0.0370	0.88
			(0.00671-0.0958)	
	Lepidostoma	24	0.0470	0.86
			(0.0193-0.0898)	
		48	0.0384	0.86
			(0.0147-0.0764)	
Chlorothalonil	Anchytarsus	24	_	
		48	4.62 (2.81–10.7)	0.41
		96	1.96 (1.25–3.2)	0.85
	Hyalella	24	2.61 (1.73-4.25)	0.76
		48	0.57 (0.28-0.99)	0.84
	Lepidostoma	24	5.08 (3.32-10.20)	0.62
		48	2.72 (1.76-4.65)	0.69

studied species. Both Anchytarsus (LC<sub>50–96 h</sub> = 1.54 µg L<sup>-1</sup>) and Hyalella (LC<sub>50–48 h</sub> = 1.77 µg L<sup>-1</sup>) had similar sensitivities to bifenthrin exposure. In the case of chlorpyrifos, Hyalella (LC<sub>50–48 h</sub> = 0.037 µg L<sup>-1</sup>) and Lepidostoma (LC<sub>50–48 h</sub> = 0.038 µg L<sup>-1</sup>) were the most sensitive species, followed by Anchytarsus (LC<sub>50–48 h</sub> = 0.079 µg L<sup>-1</sup>). For chlorothalonil, Hyalella (LC<sub>50–48 h</sub> = 0.57 µg L<sup>-1</sup>) was the most sensitive species, followed by Lepidostoma (LC<sub>50–48 h</sub> = 2.72 µg L<sup>-1</sup>) and Anchytarsus (LC<sub>50–96 h</sub> = 1.96 µg L<sup>-1</sup>).

The bifenthrin dataset used for the SSD analysis comprised LC50/  $EC_{50}$  values between 0.01 and 822  $\mu$ g L<sup>-1</sup> for 16 freshwater species (one alga, four crustaceans, eight insects and three fishes) from five different functional groups (Co, De, Om, Pp and Pr) of which only two invertebrate species corresponded to tropical detritivores (from the present study), with the rest being temperate detritivores (Table S5). Chlorpyrifos was the pesticide with the highest available information; the dataset comprised  $LC_{50}/EC_{50}$  values between 0.05 and 22,440  $\mu g \ L^{-1}$  for 49 freshwater species (four algae, 16 crustaceans, 21 insects and eight fishes) belonging to six different functional groups (Co, De, Hb, Om, Pp and Pr) of which 18 species were tested in tropical regions (including the three species of this study) and the rest in temperate zones (Table S6). For chlorothalonil, the dataset comprised LC50/EC50 values between 0.57 and 8069.3  $\mu g \ L^{\text{-1}}$  for 10 freshwater species (four algae, three crustaceans, two insects and one fish) belonging to four different functional groups (Co, De, Om and Pp; Table S7).

The SSD curves are shown in Fig. 1 and the results are summarized in Table 2. The log normal model showed the best fit for the three pesticides tested (Table S8). Bifenthrin (HC<sub>5</sub> of 0.009  $\mu$ g L<sup>-1</sup>; Fig. 1A) and chlorpyrifos (0.012  $\mu$ g L<sup>-1</sup>; Fig. 1B) were more toxic, compared to chlorothalonil (HC<sub>5</sub> of 0.523  $\mu$ g L<sup>-1</sup>; Fig. 1C). The comparison between the sensitivity of tropical and temperate species to chlorpyrifos is presented in Fig. 2. We did not find significant differences (p = 0.59) between the HC<sub>5</sub> values of tropical species (n = 18; HC<sub>5</sub> = 0.009  $\mu$ g L<sup>-1</sup>) and those of temperate species (n = 34; HC<sub>5</sub> = 0.012  $\mu$ g L<sup>-1</sup>), although the HC<sub>5</sub> estimates for tropical species tended to be lower.

# 4. Discussion

Our study demonstrates that common-use pesticides can affect several species of tropical stream detritivores, provoking the mortality of half their populations at concentrations of 0.04–2.7  $\mu$ g L<sup>-1</sup>. This is



Fig. 1. Species sensitivity distributions (SSD) and hazardous concentrations of 5% using the  $LC_{50}/EC_{50}$  values of our species and those reported for freshwater species of different functional groups. The solid line is the fitted Log Normal Model, the section in gray represent 95% confidence interval, and the dashed line red represent hazardous concentrations of 5%. A = Bifenthrin ( $\mu$ g L<sup>-1</sup>); B = Chlorpyrifos ( $\mu$ g L<sup>-1</sup>); C = Chlorothalonil ( $\mu$ g L<sup>-1</sup>). Color represents taxonomic group as follows: = Algae; = Crustaceans; = Fishes; = Insects. Shape represents functional group as follows: •= Collectors;  $\blacktriangle$  = Detritivores; = Hervibores; + = Omnivores;  $\aleph$  = Primary producers; \*= Predators.

## Table 2

Hazardous concentration for 5% of species (HC<sub>5</sub>;  $\mu$ g L<sup>-1</sup>) and their lower (95%) and upper (5%) confidence limits, calculated from species sensitivity distributions constructed for freshwater species.

Pesticide name	No. of species		HC <sub>5</sub> (95% CI)	$\mathbb{R}^2$
Bifenthrin	All species	16	0.009 (0.001-0.114)	0.98
Chlorpyrifos	All species	49	0.012 (0.003-0.0589)	0.98
	Tropical	18	0.009 (0.0005-0.469)	0.99
	Temperate	34	0.012 (0.0027-0.0603)	0.98
Chlorothalonil	All species	10	0.523 (0.0493-8.02)	0.97

important because of the key role of detritivores in stream ecosystems as main agents (together with aquatic hyphomycetes) of leaf litter decomposition, a fundamental component of stream ecosystem functioning (Graça, 2001). Information about the sensitivity of these organisms is scarce because they are not standard organisms commonly used in toxicity tests (Freitas and Rocha, 2010). However, they are highly relevant from an ecosystem perspective, and likely to be strongly affected by pesticides compared to other organisms because they can ingest pesticides not only from the water but also while feeding on leaf litter, where pesticides can accumulate (Zubrod et al., 2015; Cornejo et al., 2020a).

The scarcity of information about pesticide effects on stream organisms is particularly scarce for tropical regions (Freitas and Rocha, 2010), and our current scientific knowledge in ecotoxicology is based mostly on research from temperate systems (Gunnarsson and Castillo, 2018). This is partly due to the limited basic knowledge of benthic fauna compared to that of Europe, North America and, to a lesser extent, Australia and New Zealand (Boyero et al., 2009). Many tropical insect larval stages have not been related to adults, so species cannot be identified, and their life histories are unknown (Boyero et al., 2009). For this reason, tropical studies have often used information from well-known, standard organisms such as D. magna, which are not the most sensitive (Schäfer et al., 2013). Our three study species were more sensitive that D. magna to chlorpyrifos and chlorothalonil (but not to bifenthrin), so the consequences of these pesticides in streams where these detritivores are present would be underestimated by considering the responses of D. magna.



Fig. 2. Temperate (blue curve) and tropical (green curve) species sensitivity (SSD) for chlorpyrifos (μg L<sup>-1</sup>). The solid line is the fitted Log Normal Model (blue for temperate species and green for tropical), the grey sections represent the 95% confidence interval. Color represents taxonomic group as follows:
■ Algae; ■ Crustaceans; ■ = Fishes; ■ Insects. Shape represents functional group as follows: •= Collectors; ▲= Detritivores; ■= Hervibores; + = Omnivores; ⊠= Primary producers; \*= Predators.

As in other studies, we found no systematic differences between the sensitivity of tropical and temperate species using the SSD approach (Diepens et al., 2014; Khatikarn et al., 2016; Kwok et al., 2007; Maltby et al., 2005; Rico et al., 2010, 2011). Others had hypothesized that tropical organisms would be more susceptible to pesticide exposure than their temperate counterparts, due to the higher temperatures in the tropics and hence the higher metabolic rates of tropical organisms (Castillo et al., 1997; Peters et al., 1997). However, available evidence suggests that sensitivity to pesticides does not depend on the climatic conditions (Daam et al., 2008), but it is rather determined by species-specific traits and the toxicodynamics of the chemical (Van den Berg et al., 2019). For example, Maltby et al. (2005) found that HC<sub>5</sub> values for chlorpyrifos, fenitrothion and carbofuran tended to the lower in tropical than temperate organisms, but the differences were not statistically significant, and Kwok et al. (2007) found that temperate species were more sensitive to carbaryl, DDT, and malathion, whereas tropical species were more sensitive to chlorpyrifos.

Despite the lack of differences between the sensitivity of tropical and temperate species to pesticides, the use of these pesticides most likely has different consequences in these two zones of the world. This is because the intensive agricultural practices in tropical countries lead to higher inputs of pesticides and spread of contamination over watersheds (Daam and Van den Brink, 2010), and regulations of pesticide use are often more permissive in tropical than in temperate countries (Castillo et al., 1997). Thus, tropical streams often reach pesticide concentrations that are much higher than those in temperate streams, with more serious consequences for populations and ecosystems. Unfortunately, tropical data are still scarce in the literature, and this precludes robust tests, so our results and those of previous authors should be taken with caution and be considered as preliminary.

In conclusion, our study demonstrates a clear sensitivity of several invertebrate species that are key for the functioning of the stream ecosystem to common-use pesticides in the study area. We also show that extrapolation of results from ecotoxicological tests with *D. magna* or other common test species would underestimate the real effects of pesticides in tropical stream ecosystems and communities. We highlight the need for more ecotoxicological studies that use tropical aquatic species from different trophic levels in order to assess the (Von Fumetti and Blaurock, 2018) ecological risk of pesticide pollution in stream ecosystems.

# Funding

This work was supported by the National Secretariat for Science, Technology and Innovation of Panama (SENACYT; project APY-GC-2018B-052; contract no. 259-2018) and the Ministry of Economy and Finance of Panama (MEF; project 019910.001). AC was supported by a fellowship from SENACYT (contract no. 001-2015) and by the National Research System of Panama (SNI; Ph.D. category; contract no. 186-2018). GC was supported by a fellowship from IFARHU-SENACYT (contract no. 270-2018-1011).

### CRediT authorship contribution statement

Aydeé Cornejo: Conceptualization, Funding acquisition, Project administration, Investigation, Supervision, Methodology, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. Francisco Encina-Montoya: Funding acquisition, Investigation, Methodology, Writing - review & editing. Francisco Correa-Araneda: Funding acquisition, Investigation, Methodology, Writing - review & editing. Dalys Rovira: Funding acquisition, Investigation, Methodology, Writing - review & editing. Gabriela García: Investigation, Methodology, Data curation, Literature revision, Writing - review & editing. Carlos Nieto: Investigation, Data curation, Literature revision, Writing review & editing. Víctor Villarreal: Investigation, Methodology, Data curation, Literature revision. Nicomedes Jaramillo: Investigation, Methodology, Writing - review & editing. Edgar Pérez: Investigation, Data curation, Literature revision. Anayansi Valderrama: Investigation, Methodology, Data curation, Writing - review & editing. Javier Pérez: Supervision, Investigation, Methodology, Writing - review & editing. Luz Boyero: Conceptualization, Funding acquisition, Supervision, Investigation, Methodology, Writing - original draft, Writing - review & editing.

### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

We thank the Panamanian Ministry of Environment for their authorization to carry out the study at the PILA Station; Brenda Checa from the Ministry of Agricultural Development (MIDA), who led the RLA7019 Project, funded by the International Atomic Energy Agency (AIEA), which supported training with experts; Anyi Tuñon, Bert Kolhmann, Francisco Quesada, Jenny Bermúdez, Meyer Guevara, Milexi Molinar, Monika Springer, Silvia Echeverría-Sáenz, Tomás A. Ríos González and Yusseff Aguirre for completing the surveys for invertebrate selection; Allison Villarreal and Karina Correa for assistance with invertebrate sampling and laboratory bioassays; Jareth Roman, Rodolfo Novelo, Jose Loaiza and Mileika Santos for providing information on distribution of invertebrate species; and two anonymous reviewers for providing constructive comments on the manuscript.

# Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112226.

### References

ANAM, CATIE.2014. Plan de manejo de la cuenca del río Chiriquí Viejo.

- Bighiu, M.A., Höss, S., Traunspurger, W., Kahlert, M., Goedkoop, W., 2020. Limited effects of pesticides on stream macroinvertebrates, biofilm nematodes, and algae in intensive agricultural landscapes in Sweden. Water Res. 174, 115640 https://doi. org/10.1016/j.watres.2020.115640.
- Boyero, L., Pearson, R.G., Albariño, R.J., Callisto, M., Correa-Araneda, F., Encalada, A.C., Swan, C.M., 2020. Identifying stream invertebrates as plant litter consumers. Methods to Study Litter Decomposition. Springer, Cham, pp. 455–464.
- Boyero, L., Pearson, R.G., Dudgeon, D., Ferreira, V., Graça, M.A.S., Boulton, A.J., Chauvet, E., Yule, C.M., Albariño, R.J., Ramírez, A., Helson, J., Callisto, M., Arunachalam, M., Chará, J., Figueroa, R., Mathooko, J.M., Gonçalves Jr., J.F., Moretti, M.S., Chará, A.M., Davies, J.N., Encalada, A., Lamothe, S., Buria, L.M., Castela, J., Cornejo, A., Li, A.O.Y., M'Erimba, C., Villanueva, V.D., Zúñiga, M.C., Swan, C., Barmuta, L.A., 2012. Global patterns of distribution in stream detritivores: implications for biodiversity loss in changing climates. Glob. Ecol. Biogeogr. 21, 134–141. https://doi.org/10.1111/j.1466-8238.2011.00673.x.
- Boyero, L., Pearson, R.G., Dudgeon, D., Graça, M.A.S., Gessner, M.O., Albariño, R.J., Ferreira, V., Yule, C.M., Boulton, A.J., Arunachalam, M., Callisto, M., Chauvet, E., Ramírez, A., Chará, J., Moretti, M.S., Gonçalves Jr., J.F., Helson, J., Chará, A.M., Encalada, A., Davies, J.N., Lamothe, S., Cornejo, A., Castela, J., Aggie, O.Y., Buria, L. M., Villanueva, V.D., Zúñiga, M.C., 2011. Global distribution of a key trophic guild contrasts with common latitudinal diversity patterns, 2011 Ecology 92 (9), 1839–1848. https://doi.org/10.1890/10-2244.1.
- Boyero, L., Ramírez, A., Dudgeon, D., Pearson, R.G., 2009. Are tropical streams really different? J. N. Am. Benthol. Soc. 28 (2), 397–403. https://doi.org/10.1899/08-146.1.
- Bundschuh, M., Zubrod, J., Petschick, L., Schulz, R., 2020. Multiple stressors in auatic ecosystems: Sublethal effects of temperature, dissolved organic matter, light and a neonicotinoid insecticide on gammarids. Bull. Environ. Contam. Toxicol. 105, 345–350. https://doi.org/10.1007/s00128-020-02926-6.
- Castillo, L.E., de la Cruz, E., Ruepert, C., 1997. Ecotoxicology and pesticides in tropical aquatic ecosystems of Central America. Environ. Toxicol. Chem. 16, 41–51. https:// doi.org/10.1002/etc.5620160104.
- Cheshire, K., Boyero, L., Pearson, R.G., 2005. Food webs in tropical Australian streams: shredders are not scarce. Freshw. Biol. 50, 748–769. https://doi.org/10.1111/ J.1365-2427.2005.01355.X.
- Cornejo, A., Pérez, J., Alonso, A., López-Rojo, N., Monroy, S., Boyero, L., 2020a. A common fungicide impairs stream ecosystem functioning through effects on

#### A. Cornejo et al.

#### Ecotoxicology and Environmental Safety 216 (2021) 112226

aquatic hyphomycetes and detritivorous caddisflies. J. Environ. Manag. 263, 110425 https://doi.org/10.1016/j.jenvman.2020.110425.

- Cornejo, A., Pérez, J., López-Rojo, N., Tonin, A.M., Rovira, D., Checa, B., Jaramillo, N., Correa, K., Villarreal, A., Villarreal, V., García, G., Pérez, E., Ríos González, T.A., Aguirre, Y., Correa-Araneda, F., Boyero, L., 2020b. Agriculture impairs stream ecosystem functioning in a tropical catchment. Sci. Total Environ. 745. https://doi. org/10.1016/j.scitotenv.2020.140950.
- Cornejo, A., Tonin, A.M., Checa, B., Tuñon, A.R., Pérez, D., Coronado, E., González, S., Ríos, T., Macchi, P., Correa-Araneda, F., Boyero, L., 2019. Effects of multiple stressors associated with agriculture on stream macroinvertebrate communities in a tropical catchment. PLoS One 14 (8), e0220528. https://doi.org/10.1371/journal. pone.0220528.
- Cummins, K.W., Klug, M.J., 1979. Feeding ecology of stream invertebrates. Annu. Rev. Ecol. Syst. 10 (1), 147–172. https://doi.org/10.1146/annurev.
- Cummins, K.W., Wilzbach, M.A., Gates, D.M., Perry, J.B., Taliaferro, W.B., 1989. Shredders and riparian vegetation. BioScience 39 (1), 24–30. https://doi.org/ 10.2307/1310804.
- Daam, M.A., Rico, A., 2018. Freshwater shrimps as sensitive test species for the risk assessment of pesticides in the tropics. Environ. Sci. Pollut. Res. 25, 13235–13243. https://doi.org/10.1007/s11356-016-7451-1.
- Daam, M.A., Van den Brink, P.J., 2010. Implications of differences between temperate and tropical freshwater ecosystems for the ecological risk assessment of pesticides. Ecotoxicology 19, 24–37. https://doi.org/10.1007/s11356-016-7451-1.
- Daam, M.A., Crum, S.J.H., Van den Brink, P.J., Nogueira, A.J.A., 2008. Fate and effects of the insecticide chlorpyrifos in outdoor plankton- dominated microcosms in Thailand. Environ. Toxicol. Chem. 27, 2530–2538. https://doi.org/10.1007/ s10646-009-0402-6.
- Diepens, N.J., Pfennig, S., van den Brink, P.J., Gunnarsson, J.S., Ruepert, C., Castillo, L., 2014. Effect of pesticides used in banana and pineapple plantations on aquatic ecosystems in Costa Rica. J. Environ. Biol. 35, 73–84.
- ETESA, 2008. Resumen Técnico análisis regional de crecidas máximas de Panamá, periodo 1971-2006. República de Panamá, Panamá.
- Freitas, E.C., Rocha, O., 2010. Acute toxicity tests with the tropical cladoceran *Pseudosida* ramosa: the importance of using native species as test organisms. Arch. Environ. Contam. Toxicol. 60 (2), 241–249. https://doi.org/10.1007/s00244-010-9541-2.
- Gibbs, H.K., Ruesch, A.S., Achard, F., Clayton, M.K., Holmgren, P., Ramankutty, N., Foley, J.A., 2010. Tropical forests were the primary sources of new agricultural land in the 1980s and 1990s. Proc. Natl. Acad. Sci. U.S.A. 107, 16732–16737. https://doi. org/10.1073/pnas.0910275107.
- Graça, M.A.S., 2001. The role of invertebrates on leaf litter decomposition in streams—A review. Int. Rev. Hydrobiol. 86, 383–393.
- Gunnarsson, J.S., Castillo, L.E., 2018. Ecotoxicology in tropical regions. Environ. Sci. Pollut. Res. 25, 13203–13206. https://doi.org/10.1007/s11356-018-1887-4.
- Hlina, B.L., Birceanu, O., Robinson, C.S., Dhiyebi, H., Wilkie, M.P., 2019. In Review. Seasonal variation in the sensitivity of invasive sea lampreys to the Lampricide TFM: Importance of energy reserves and temperature. N. Am. J. Fish. Manag.
- Khatikarn, J., Satapornvanit, K., Price, O.R., Van den Brink, P.J., 2016. Effects of triclosan on aquatic invertebrates in tropics and the influence of pH on its toxicity on microalgae. Environ. Sci. Pollut. Res. 25, 13244–13253. https://doi.org/10.1007/ s11356-016-7302-0.
- Kwok, K.W.H., Leung, K.M.Y., Chu, V.K.H., Lam, P.K.S., Morritt, D., Maltby, L., Brock, T. C.M., Van den Brink, P.J., Warne, M.St.J., Crane, M., 2007. Comparison of tropical and temperate freshwater species sensitivities to chemicals: implications for deriving safe extrapolation factors. Integr. Environ. Assess. Manag. 3, 49–67.

- López-Rojo, N., Pozo, J., Pérez, J., Basaguren, A., Martínez, A., Tonin, A.M., Correa-Araneda, F., Boyero, L., 2019. Plant diversity loss affects stream ecosystem multifunctionality. Ecology 100, e02847. https://doi.org/10.1002/ecy.2847.
- Maltby, L., Blake, N., Brock, T.C.M., Van den Brink, P.J., 2005. Insecticide species sensitivity distributions: importance of test species selection and relevance to aquatic ecosystems. Environ. Toxicol. Chem. 24, 288–379. https://doi.org/10.1897/04-025r.1.
- MIDA, 2016. Enfoque integral de contaminantes químicos y biomonitoreo en la cuenca alta del río Chiriquí Viejo. Resultados Preliminares, Proyecto ARCAL RLA 7019 Desarrollo de indicadores para determinar el efecto de plaguicidas, metales pesados y contaminantes emergentes en ecosistemas acuáticos importantes para la agricultura y agroindustria. República de Panamá, Panamá.
- Posthuma, L., Suter II, W.G., Traas, T.P., 2002. Chapter 1. General introduction to species sensitivity distributions. In: Posthuma, L., Suter II, G.W., Traas, T.P. (Eds.), Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, FL, USA, pp. 3–9.
- Rasmussen, J.J., Reiler, E.M., Carazo, E., Matarrita, J., Munoz, A., Cedergreen, N., 2016. Influence of rice field agrochemicals on the ecological status of a tropical stream. Sci. Total Environ. 542, 12–21. https://doi.org/10.1016/j.scitotenv.2015.10.062.
- Rico, A., Geber-Corréa, R., Souto, P.C., Garcia, M.V.B., Waichman, A.V., Van, den Brink, P.J., 2010. Effect of parathion-methyl on Amazonian fish and freshwater invertebrates: a comparison of sensitivity with temperate data. Arch. Environ. Contam. Toxicol. 58, 765–771. https://doi.org/10.1007/s00244-009-9409-5.
- Rico, A., Waichman, A.V., Geber-Corréa, R., Van den Brink, P.J., 2011. Effects of malathion and carbendazim on Amazonian freshwater organisms: comparison of tropical and temperate species sensitivity distributions. Ecotoxicology 20, 625–634. https://doi.org/10.1007/s10646-011-0601-9.
- Schäfer, R.B., van den Brink, P.J., Liess, M., 2011. Chapter 6: impacts of pesticides on freshwater ecosystems. In: Sánchez-ayo, Francisco, van den Brink, Paul J., Mann, Reinier M. (Eds.), Ecological Impacts of Toxic Chemical, pp. 111–137.
- Schäfer, R.B., Gerner, N., Kefford, B.J., Rasmussen, J.J., Beketov, M.A., de Zwart, D., Liess, M., von der Ohe, P.C., 2013. How to characterize chemical exposure to predict ecologic effects on aquatic communities? Environ. Sci. Technol. 47, 7996–8004. https://doi.org/10.1021/es4014954.
- Thorley, J., Schwarz, 2018. ssdtools: an R package to fit species sensitivity distributions. J. Open Source Softw. 3 (31), 1082. https://doi.org/10.21105/joss.01082.
- U.S. Environmental Protection Agency, (U.S. EPA), 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition, EPA-821-R-02–012, Office of Water (4303T), 1200 Pennsylvania Avenue, NW, Washington, D.C., 20460.
- Van den Berg, S., Baveco, H., Butler, E., De Laender, F., Focks, A., Franco, A., Randal, C., van den Brink, P.J., 2019. Modeling the sensitivity of aquatic macroinvertebrates to chemicals using traits. Environ. Sci. Technol. 53, 6025–6034. https://doi.org/ 10.1021/acs.est.9b00893.
- Von Fumetti, S., Blaurock, K., 2018. Effects of the herbicide Roundup® on the metabolic activity of Gammarus fossarum Koch, 1836 (Crustacea; Amphipoda). Ecotoxicology 27, 1249–1260. https://doi.org/10.1007/s10646-018-1978-5.
- Yule, C.M., Boyero, L., Marchant, R., 2010. Effects of sediment pollution on food webs in a tropical river (Borneo, Indonesia). Mar. Freshw. Res. 61, 204–213. https://doi.org/ 10.1071/MF09065.
- Zubrod, J.P., Englert, D., Wolfram, J., Wallace, D., Schnetzer, N., Baudy, P., Konschak, M., Schulz, R., Bundschuh, M., 2015. Waterborne toxicity and diet-related effects of fungicides in the key leaf shredder *Gammarus fossarum* (Crustacea: Amphipoda). Aquat. Toxicol. 169, 105–112. https://doi.org/10.1016/j. aquatox.2015.10.008.