TOXICITY OF ORGANOPHOSPHATE AND PYRETHROIDS PESTICIDES ON THE TROPICAL FRESHWATER SHRIMP *XIPHOCARIS ELONGATA*

By

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DEDICATION

I dedicate this thesis to my mother, Marilyn Pérez Villa, thank you so much for believing in me and my ability to succeed in this endeavor, especially at those times when I doubted myself. When I hit the deepest of frustrations, you made sure I could find the way forward again. I also dedicate this work to all my family, especially my grandmother Cecilia Villa and my aunt Jeanette Pérez for their love, affection and support.

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ABSTRACT

Freshwater environments are highly dynamics but are subject to natural and anthropogenic influences. Although lotic ecosystems have resilience to deal with natural disturbance, the additional pressure from anthropogenic stresses can often lead to irreversible changes. Particularly, urban activities have resulted in increased anthropogenic discharge into freshwater environments with significant effects on organisms. The main objective of this thesis was to evaluate the toxicity of the freshwater shrimp *Xiphocaris elongata* to synthetic pyrethroid and organophosphate pesticides. Experiments were conducted in order to determine the effects of permethrin, malathion and glyphosate on different endpoints.

Urban activities are known to decrease the abundance of organisms living in freshwater environments. For this reason, we evaluated the population status of *X. elongata* between urban and forest streams. The results showed that urban areas negative impact the abundance of the freshwater shrimp. These findings are consistent with symptoms associated with the urban stream syndrome. As part of urban activities, the uncontrolled application of organophosphate and pyrethroids pesticides like glyphosate, malathion, and permethrin has become a growing concern due to their ability to exert an adverse effect on non-targeted organisms. This research found that acute toxicity for permethrin, malathion and glyphosate progressively increases as the concentration of exposure pesticide increased.

In natural environments, decaying leaf material may accumulate contaminants, including insecticides and herbicides. Thus, shredder shrimp can be exposed to these types of pesticide by leaf litter material. For this reason, we evaluated if the shredder shrimp display preference for feeding on different leaf species and leaf size area, while also assessing their consumption of leaves contaminated with pesticides. We also measured the acetylcholinesterase activity as a possible biomarker. The size area and plant species more appropriate for future toxicological studies is *Spathodea campanulata* leaves with a size area of 0.65 cm^2. This thesis showed that sublethal concentrations of malathion and permethrin in leaves have a significant effect on ingestion rates. Immunofluorescence shown a decline in acetylcholinesterase activity when sublethal dose of malathion and permethrin increases. This research highlights the potential of *X. elongata* as bioindicator to assess the risk of pesticide contamination in freshwater environments.

Keywords: *Aquatic toxicology, pesticides, Puerto Rico, shredder shrimp, Xiphocarididae*

CHAPTER I

GENERAL INTRODUCTION

Freshwater environments are highly dynamics but are subject to natural and anthropogenic impacts. These influences can cause physical, chemical, and biological perturbations, with significant effects on composition and abundance of organism living in freshwater environments. Although lotic ecosystems have resilience to deal with natural disturbance, the additional pressure from anthropogenic stresses can often lead to irreversible changes (Walsh et al. 2005). Particularly, urban and industrial activities have resulted in increased anthropogenic discharge into freshwater environments with significant effects on organisms. Freshwater shrimp in tropical islands have been used extensively as biological indicators of stream health and much is known about their ecology (Covich et al. 2009; 2003; 1996). Freshwater shrimp in the tropics show an amphidromous life cycle where adults reproduce in high elevations of the watershed, then the larvae spend time in the estuary until they metamorphose and migrate upstream (Kikkert et al. 2009). Freshwater shrimp are also important processor of allochthonous carbon inputs and food source for other aquatic and terrestrial organisms (Baxter et al. 2005; Covich & McDowell, 1996; Cummins et al. 1989).

In natural environments, decaying leaf material may accumulate contaminants, including insecticides and herbicides present in surface water (Englert et al. 2017; Kreutzweiser et al. 2007; Rasmussen et al. 2012). Thus, freshwater shrimp can be exposed to contaminants by leaf litter material (Wilding & Maltby, 2006; Forrow & Maltby, 2000). A better understanding of the relationships between freshwater shrimps and stream pollution could offer not only an opportunity to study population dynamics in urban streams, but also a chance to evaluate the impacts of contaminants on molecular and behavioral level.

Pollution in freshwater environments

The words "contaminant" and "pollutant" are often used interchangeably when discussing chemical, physical or biological alterations to environment. According to Chapman (2007), a contaminant is a substance present where it should not be or above normal background concentrations, while a pollutant is a contaminant that produce an adverse biological and ecological effects. Chemicals introduced into the environment may be available to organisms depending on their structure and degradation factors (Chapman, 2007). A variety of contaminants can impact aquatic environments including pesticides, pharmaceuticals, personal care products, and chemicals from industrial activity. These contaminants can come from point sources, including urban and industrial wastewater effluent, and non-point sources including stormwater and lawn care services (Carpenter et al. 1998).

Pesticides differ from other contaminants in that they are developed with the intention of exert toxic effects on target organisms (Lushchak et al. 2018). But their toxicity is usually not restricted to the location where they are applied. They can reach other places impacting different non-target organisms. Pesticides have the potential to enter aquatic environments from direct application, terrestrial runoff, and wind dispersal (Relyea & Hoverman, 2006). However, chemical analyses and quantification of pesticides is usually limited in freshwater environments. Some assessment studies have identified different types of pesticides including insecticides like malathion, permethrin, and endosulfan as well as the herbicides atrazine and glyphosate (Ronco et al. 2016; Hoffman et al. 2000; Laabs et al. 2002). These pesticides are not designed or developed to be apply in freshwater environments, however they are present in some streams.

Organophosphate and pyrethroids pesticides in freshwater environments

Pesticides can be divided into four main groups: organochlorines, organophosphates, carbamates and pyrethroids. The response of organisms to these pesticides will depend on concentration and mode of action of the chemical. Organophosphate compounds (OPs) included different insecticides used widely in residential, urban, and agricultural zones. The toxicity of organophosphate insecticides is related to its leaving group and the phosphorus ligands (Landis et al. 2018). In addition, the toxicity of these compounds is due to their ability to bind to the amino acid serine, making it incapable of participating in catalytic reactions within an enzyme blocking the active site (Landis et al. 2018).

A common organophosphate insecticide used to control mosquitoes and fruit flies is malathion (Fig. 1.1). As an organophosphate compound, malathion can inhibit acetylcholinesterase enzyme (AChE) in organisms. Acetylcholinesterase is a critical enzyme for the normal function of the nervous system (Lionetto et al. 2011). The inhibition of this enzyme can result in the accumulation of the neurotransmitter acetylcholine in the synaptic gap leading to disruption of the nervous system. Previous studies have found that malathion is highly toxic to many fish, crustaceans and invertebrates (Bihel & Callaghan, 2004; Printes & Callaghan, 2004; Kumar & Jawahar, 2013). For these reasons, the exposure of malathion to non-target freshwater organisms is of interest to aquatic toxicologists.

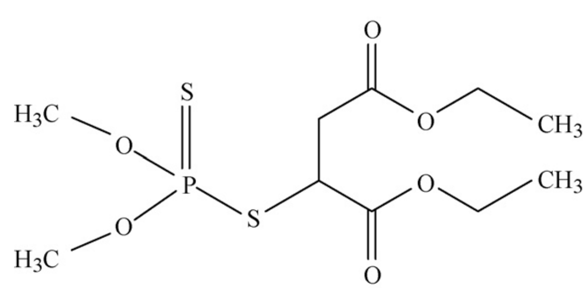

Fig. 1.1 –Molecular structure of malathion.

Glyphosate is an organophosphate pesticide commonly used to control plants and grasses (Fig. 1.2). This pesticide does not have the same mode of action of insecticides. The primary mode of action of glyphosate is confined to the shikimate pathway that links primary and secondary metabolisms (Mensah et al. 2015). Specifically, glyphosate inhibits the activity of the enzyme 5-enolpyuvylshikimate-3-phosphate synthase (EPSPS) found in plants (Stenersen, 2004). Due to its toxicity to humans, it has been recently banned in several countries (Van Bruggen et al. 2018). The exposure of glyphosate to non-target aquatic organisms is of great concern because of their extensive use, high water solubility and slow degradation rate in freshwater environments.

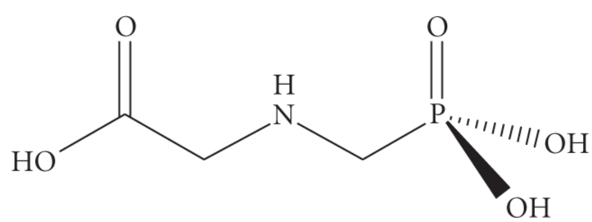

Fig 1.2 –Molecular structure of glyphosate.

Another widely used main group of pesticides are synthetic pyrethroids. These pesticides are used to control a wide range of insects. Pyrethroids, like other types of insecticides, interfere with the function of the nervous system. The mode of action of these insecticides are related to the sodium channels in nerve membranes and inhibition of acetylcholinesterase enzymes (Oliveira et al. 2012; Cox, 1998). Pyrethroid insecticides induce a continuous series of nerve impulses which disrupts the normal functioning of the nervous system. A common synthetic pyrethroid used in agricultural and residential zones to control insects is permethrin (Fig 1.3). The application of this insecticide is increasing as it is used in mosquito control programs (US Environmental Protection Agency, 2005).

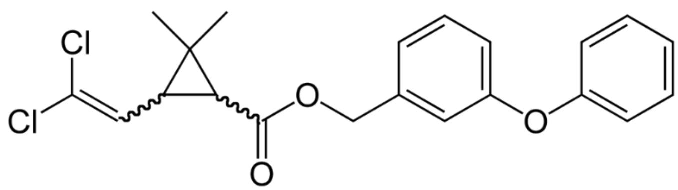

Fig 1.3 –Molecular structure of permethrin

Toxicological data for permethrin, malathion and glyphosate are available for a variety of aquatic model organisms, but there is a need of information applicable to tropical regions (Dam & Van den Brink, 2010; Gunnarsson & Castillo, 2018). For this reason, the increase use of pesticides in the tropics has become a growing concern due to their potential adverse effects on non-target aquatic organisms. In Puerto Rico, organophosphate pesticides like glyphosate and malathion, and synthetic pyrethroids like permethrin can be found in freshwater environments (Buttermore et al. 2018; Santiago et al. 2016).

Aims and outline of the thesis

The main goals of this thesis were to evaluate the toxicity of the tropical freshwater shrimp *Xiphocaris elongata* (Guérin-Ménevill, 1856) to three commonly used pesticides. To achieve that, the effects of permethrin, malathion and glyphosate were assessed at different endpoints: survival, feeding behavior and biochemical biomarker. This thesis was divided in five chapters:

- Chapter I – General introduction
- Chapter II – Population status of the tropical freshwater shrimp *Xiphocaris elongata* in urban and forest streams in Puerto Rico
- Chapter III – Acute toxicity of malathion, permethrin and glyphosate on the tropical freshwater shrimp *Xiphocaris elongata*

- In Chapter IV – Effect of particle size and pesticide contamination on preference and ingestion rates by the tropical freshwater shrimp *Xiphocaris elongata*
- In Chapter V – General conclusion

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CHAPTER II

POPULATION STATUS OF THE TROPICAL FRESHWATER SHRIMP *XIPHOCARIS ELONGATA* IN URBAN AND FOREST STREAMS IN PUERTO RICO

Abstract

Most of the human population now lives in cities and understanding their impact to freshwater environments is an important issue. Streams in cities face many environmental challenges that have been described in the concept of Urban Stream Syndrome. This concept illustrates biological, hydrological, chemical and physical stressors commonly found in urban streams. In tropical streams, there are a variety of aquatic organisms including freshwater shrimp, fish, and macroinvertebrates that can be impacted by urban activities. Particularly, freshwater shrimp are vulnerable to physical, chemical and ecological impacts of urban activities. For this reason, freshwater shrimp have been used as biological indicators of stream health and much is known about their ecology in tropical streams. Shredder shrimp are particularly important as they connect all functional groups of food webs. In particular, the shredder shrimp *Xiphocaris elongata* plays a fundamental role in organic matter decomposition. The objectives of this study were: 1) to determine the population status of *X. elongata* and 2) to identify differences in abundance of *X. elongata* between urban and forest streams. Our results showed that highly urbanized areas have significantly less abundance of the shredder shrimp *X. elongata* in comparison with medium or low urban reach in the Río Piedras and Río Sabana watersheds. This study also showed that physicochemical and geomorphological variables are important environmental factors that influence the abundance of *X. elongata* in Puerto Rico streams.

Keywords: *shredder shrimp, tropical stream, urban watershed, Xiphocarididae*

Introduction

One of the most important socioecological concerns of the 21st century is the rise of urban areas as the dominant geographical context on Earth (World Watch Institute, 2007). Urban activities can have important and sometimes irreversible effects on the natural world. Many streams in urban areas face environmental challenges in the form of biological, hydrological, chemical, and physical stressors as described by the Urban Stream Syndrome (Walsh et al. 2005). According to the Urban Stream Syndrome, one of the primary drivers of degraded conditions in urban streams is the impervious surface cover which inhibits the infiltration of water into the soil, increasing runoff directly into drainages and streams (Arnold & Gibbons, 1996). Increased runoff in streams causes a 'flashy' hydrology with increased in frequency and magnitude of flooding (Paul & Meyer, 2001). This response can lead to stream bank erosion and sedimentation of stream channels (Arnold & Gibbons, 1996).

In addition to changes in hydrology, urban streams can become contaminated with different chemicals, including pesticides, pharmaceuticals, personal care products, and industrial residues (Paul & Meyer, 2001; Carpenter et al. 2011). These contaminants can come from point sources, including urban and industrial wastewater effluent, and non-point sources like stormwater and lawn care products (Carpenter et al. 1998). Natural tropical streams tend to have high densities of aquatic organisms including freshwater shrimp, fish, and other macroinvertebrates that are affected by urban activities. However, there is a need to document these effects. This is particularly needed for freshwater shrimp, which are vulnerable to the physical, chemical and ecological stressors of urban activities (Pérez-Reyes et al. 2016). For this reason, freshwater shrimp has been used as biological indicators of stream health and much is known about their ecology in tropical streams (Covich et al. 2003; 2006; 2009). In Puerto Rico, a total of 17 species of freshwater shrimp from three families have been described (Pérez-Reyes et al. 2013). All freshwater shrimp in the island show an amphidromous life cycle where adults reproduce in high elevations of the watershed, then the larvae spend time in the estuary until they metamorphose and migrate upstream (Kikkert et al. 2009).

Among the species described for Puerto Rico, the shredder *Xiphocaris elongata* (Guérin-Méneville, 1856) plays a fundamental role in organic matter decomposition. Shredder shrimp have an important role as organisms that connect all functional groups of food webs (Covich & McDowell, 1996). The objectives of this study were: 1) to determine the population status of *X. elongata* and 2) to identify differences in abundance of *X. elongata* between urban and forest streams. We hypothesized that there would be a decreased abundance of *X. elongata* in watersheds with different urban gradients due to alterations in their habitat. We compared the abundance of *X. elongata* between Río Piedras and Río Sabana watersheds, and between different urban impacted reaches (low, medium, and high). Also, we compared the abundance of *X. elongata* between different rainfall season [Low Rainfall- (LRF) and High Rainfall- (HRF)]. We expected to find lower abundance of freshwater shrimp in watersheds with a higher and medium urban land cover because of changes in physicochemical variables.

Materials and Methods

Study sites

Río Piedras and Río Sabana watersheds were selected in order to describe and compare the population status of the freshwater shrimp *Xiphocaris elongata*. Río Sabana and Río Piedras watersheds represent low and high level of urbanization based on the percentage of urban cover, respectively. In each watershed, three sampling locations were selected from the river mouth to the headwaters to maximize the range of habitat types (i.e., pools, runs, and riffles) in different urban intensities. At each site, two 10 m sampling reaches were randomly selected to sample freshwater shrimp and measure their physicochemical parameters (a total of three sampling location in each watershed).

Sampling locations within the watersheds were classified as low (LU), medium (MU) and high urban (HU) reaches. High urban (HU) reaches were defined as an area adjacent to a densely settled census blocks with a total population between 2,500 to 50,000 (US Census Bureau, 2012). Medium urban (MU) or rural reaches consisted of census blocks with a population between 2,500 to 1,000 (US Census Bureau, 2012). Finally, low urban (LU) or natural reaches were defined as an area that appeared to be minimally altered by human actions and have less than 1,000 inhabitants (Table 2.1).

Río Sabana watershed (Fig. 2.1) is located in the northeastern area of Puerto Rico. The upper headwaters of the watershed are managed by the U.S. Forest Service to sustain the riparian forest cover (Heartsill-Scalley & López-Marrero, 2014). However, for hundreds of years humans have impacted the middle and lower part (Thomlison & Rivera, 2000). The land cover is composed of 71.4 % of forest, 15.8 % pasture and 8.8 % urban areas (Heartsill-Scalley & López-Marrero, 2014).

Río Piedras watershed (Fig. 2.2) have the highest level of urban development in the island. It has a population between 2 – 2.5 million, making it the second largest city in the Caribbean region (Lugo et al. 2011; Ramírez et al. 2014). This watershed drains most of San Juan Metropolitan area (De Jesús-Crespo & Ramírez, 2011; Ramírez et al. 2009). The main water source of the Río Piedras is located at about 70 m above sea level where the flow is regulated by Lago Las Curías (Pérez-Reyes et al. 2016). This river flows into to Caño Martin Peña where it enters into the San Juan Bay Estuary, but before it passes through different urban areas of San Juan

(Lugo et al. 2011; Pérez-Reyes et al. 2016). The land cover in this watershed was composed of 55.4 % urban areas, 15.3 % pastures, and 26.4 % forest (Ramos-González, 2014).

Physicochemical variables

In each watershed, river habitat was quantified through direct measurements of physical characteristics of the pools (length, width, depth, substrate composition, and pool canopy cover) at six sampling stations (two per sampling location) with low, medium and high urban reaches areas. Substrate composition of pools were determined using four samples of one hundred rocks that were chosen randomly in an area of 0.5 m^2. A gravelometer were used to classify the substrate. Canopy cover were estimated using a spherical densitometer. Five physicochemical variables were measured (temperature, pH, conductivity, dissolved oxygen and turbidity) using a Hydrolab DS5X multiparameter sonde previously calibrated following manufacturer instructions.

Freshwater shrimp sampling

Freshwater shrimp were collected using an electrofishing backpack (Model 12-B, Smith-Root, Vancouver, Washington, USA). Collections consisted of five upstream electrofishing passes in each sampling reach (10 m). Hand nets were used to collect the organisms. The habitats sampled included riffles, runs, pools, and aquatic vegetation. The cephalothorax length (CL) of each shrimp was measured from the post-orbital region to the end of the carapace with a dial caliper (0.01 mm precision). The measurement from the tip of the rostrum was not used because the length among *Xiphocaris elongata* varies depending on the presence of fish predators (Ocasio-Torres et al. 2014; Covich et al. 2009). Catch per unit effort (CPUE) was calculated for one-year sampling (October 2018– September 2019). Catch per unit effort represents the quantity of shrimp divided by the sampling effort (e.g. number of passes in each sampling location).

Statistical analyses

The environmental and physicochemical variables were compared among study sites for both watersheds using one-way ANOVA analysis. Two-way ANOVA were used to compare the catch per unit effort among streams, urban reaches, and between two rainfall periods (low rainfall season (LRF) – December to March and high rainfall season (HRF) – April to November). Nonmetric multidimensional scaling (NMDS) analysis was used to describe the relationship between the environmental variables and *X. elongata* abundance. The Bray-Curtis Dissimilarity Index was used as the distance variable for NMDS ordination (Bray & Curtis, 1957).

Results

Physicochemical variables

In Río Sabana watershed, one-way ANOVAs for physicochemical parameters showed significant differences in temperature, pH, turbidity and conductivity among sampling stations (Table 2.2). Our results showed an increased stream temperature from headwaters to river outlet. The lowest mean temperature was recorded in low urban reach (21.3±0.03 ºC), while medium and high urban reaches had mean values of 21.7±0.06 ºC and 22.3±0.03 ºC, respectively (Table 2.2**)**. Significant differences in pH were observed among reaches (Table 2.2). Low urban reach had the lowest pH mean value (7.43±0.03) while the highest mean values were observed in the high urban reach (7.58±0.03). Río Sabana watershed also had significant differences in turbidity and conductivity among study reaches (Table 2.2).

One-way ANOVAs for physicochemical parameters in Río Piedras watershed showed significant differences in temperature, pH, turbidity, conductivity and dissolved oxygen (Table 2.3). The lowest mean temperature was recorded in the low urban reach (22.4±0.04 ºC), while medium and high urban sites had mean values of 23.8±0.03 ºC and 23.9±0.03 ºC, respectively

(Table 2.3). Similarly, the lowest conductivity values were also found in low urban sites (330.0±1 $\mu S.cm^{-1}$) while the highest values were observed in high urban areas (354.0±1 $\mu S.cm^{-1}$). Significant differences in pH values were also observed among study sites (Table 2.3). The low urban sites had the lowest pH mean value (7.7±0.03) while the highest values were observed in high urban sites (7.9±0.03).

One-way ANOVAs for physicochemical parameters showed significant differences between Río Piedras and Río Sabana watersheds in temperature, pH, turbidity, conductivity and dissolved oxygen (Table 2.4). Our results showed that stream water temperatures are higher in Río Piedras watershed for all sampling sites in comparison to Río Sabana watershed. The lowest stream water temperature was observed in low urban reach in Río Sabana (21.3±0.03 ºC) while the highest water temperature was observed in Río Piedras high urban reach (23.9±0.03 ºC). Significant differences in turbidity were observed among watersheds (Table 2.4). The low urban site in Río Sabana had the lowest concentrations in turbidity with a mean value of 0.1±0.0004 NTU, while the highest mean value was observed in Río Piedras watershed (0.2 ± 0.001 NTU). Water conductivity and pH values also varied significantly among watersheds (Table 2.4). One-way ANOVAs comparing pool canopy, pool depth, and substrate index among Río Piedras and Río Sabana watersheds, and between urban reach showed statically significant differences (Table 2.5 – 2.7).

Freshwater shrimp sampling

Significant differences were found in catch per unit efforts between Río Piedras and Río Sabana watersheds (Two-way ANOVA, $F_{1,66}$=42.8; $p < 0.001$). Urban reaches in Río Piedras and Río Sabana watershed demonstrated also statistically significant differences in CPUE (Two-way ANOVA, $F_{2,66}$=15.9; $p < 0.001$) (Fig. 2.3; Table 2.8). In addition, statistically differences were found between the combination of urban reaches within Río Piedras and Río Sabana watersheds (Two-way ANOVA, $F_{2,66}$=9.3; $p < 0.01$) (Fig. 2.3; Table 2.8). Our results showed that high urban reaches in Río Piedras (4.4 ± 0.6 CPUE) and Río Sabana (7.4 ± 0.5 CPUE) had significantly different mean catch per unit effort. Similar results were found for medium and low urban reaches (Fig. 2.3). Finally, CPUE between high rainfall season and low rainfall season showed no significant difference for Río Sabana watershed (Fig. 2.4; Table 2.9). However, in the Río Piedras watershed significant differences were found between rainfall seasons (Two-way ANOVA, $F_{1,30}$=50.1; $p < 0.001$) (Fig. 2.4; Table 2.10).

Multivariable Analysis

Non-Metric Multidimensional Scaling ordination of *Xiphocaris elongata* abundance in Río Piedras and Río Sabana watersheds generated two major axes that explained most of the variation. In Río Piedras watershed, axis 1 and 2 were highly related to turbidity (0.9) and pool depth (0.9), respectively (Fig. 2.5). Turbidity represented the environmental variable that explained most of the variation in *Xiphocaris elongata* abundance in Río Piedras watershed. The environmental variable that best explained the variation on axis 2 was pool depth. Study sites with deepest pools were observed in high (0.53±0.05m) and low (0.42±0.04m) urbanized areas. Other physicochemical variables like pH and temperature were also important factors with direct influence in *X. elongata* abundance in this watershed. In Río Sabana watershed, Axis 1 and 2 were highly related to temperature (0.9) and pool canopy cover (0.9), respectively (Fig. 2.5). Water temperature was the environmental variable that best explained the variation of abundance in this watershed. However, pH and turbidity variables can also influence *X. elongata* abundance in Río Sabana watershed.

Discussion and conclusions

Urban activities are known to decrease the abundance of organisms living in freshwater environments (De Jesús-Crespo & Ramírez, 2011; Urban et al. 2006; Brasher, 2003). However, it is not clear which environmental and anthropogenic variables are most important for the observed decrease. This study found that urban reaches negatively affect the abundance of the freshwater shrimp *Xiphocaris elongata*. This result confirms a previous study that found a decrease in species richness and abundance of decapod communities in highly urbanized watersheds in Puerto Rico (Pérez-Reyes et al. 2016). We also found significant differences in catch per unit effort between high rainfall season (HRF) and low rainfall season (LRF) in Río Piedras watershed. This difference could be related to extremely high stream discharge events that commonly occur in tropical urban streams. These events can scour stream channels and displace freshwater shrimp affecting their densities. However, in forested streams freshwater shrimp take advantage of high discharge events to transport their larvae downstream to the estuary to complete their life cycle (Bauer, 2013). For Río Sabana watershed, no significant differences were found between HRF and LRF season. Similar results have been found in forested watersheds were the abundance of decapod communities showed no variations between rainfall seasons (Pérez-Reyes et al. 2016).

The abundance of *X. elongata* in Río Sabana watershed was best explained by temperature and pool canopy cover. Warmer temperatures were found in highly urbanized areas with lower canopy cover. Previous studies showed that higher water temperatures in urban stream environments are associated with reduced canopy cover (Dugdale et al. 2018; Brasher, 2003). Also, the transformation of natural areas to impervious surface cover, like roads and channels, can facilitate the transfer of heat to freshwater environments due to the reductions in canopy cover (Kaushal & Belt, 2012). Higher temperature in streams can increase decomposition rates and organism metabolism reducing dissolved oxygen in the water. In Río Piedras watershed, *X. elongata* abundance were best explained by turbidity and pool depth. In urban areas, higher turbidities were found in deeper pools. These results are consistent with symptoms associated with the urban stream syndrome (Walsh et al. 2005). A previous study in Puerto Rico found that increases in turbidity levels can have a negative effect on the migratory behavior of *X. elongata* (Kikkert et al. 2009). In summary, it is evident that in tropical urban streams the effects of human activities are affecting water quality, hydrology, geomorphology, and freshwater organisms. This study found that highly urbanized reach has less abundance of the shredder shrimp *Xiphocaris elongata* in comparison with reaches classified as medium or low urban in Río Piedras and Río Sabana watershed. Our results demonstrate that physicochemical and geomorphological variables are important environmental factors that influence the abundance of *X. elongata* in Puerto Rico streams.

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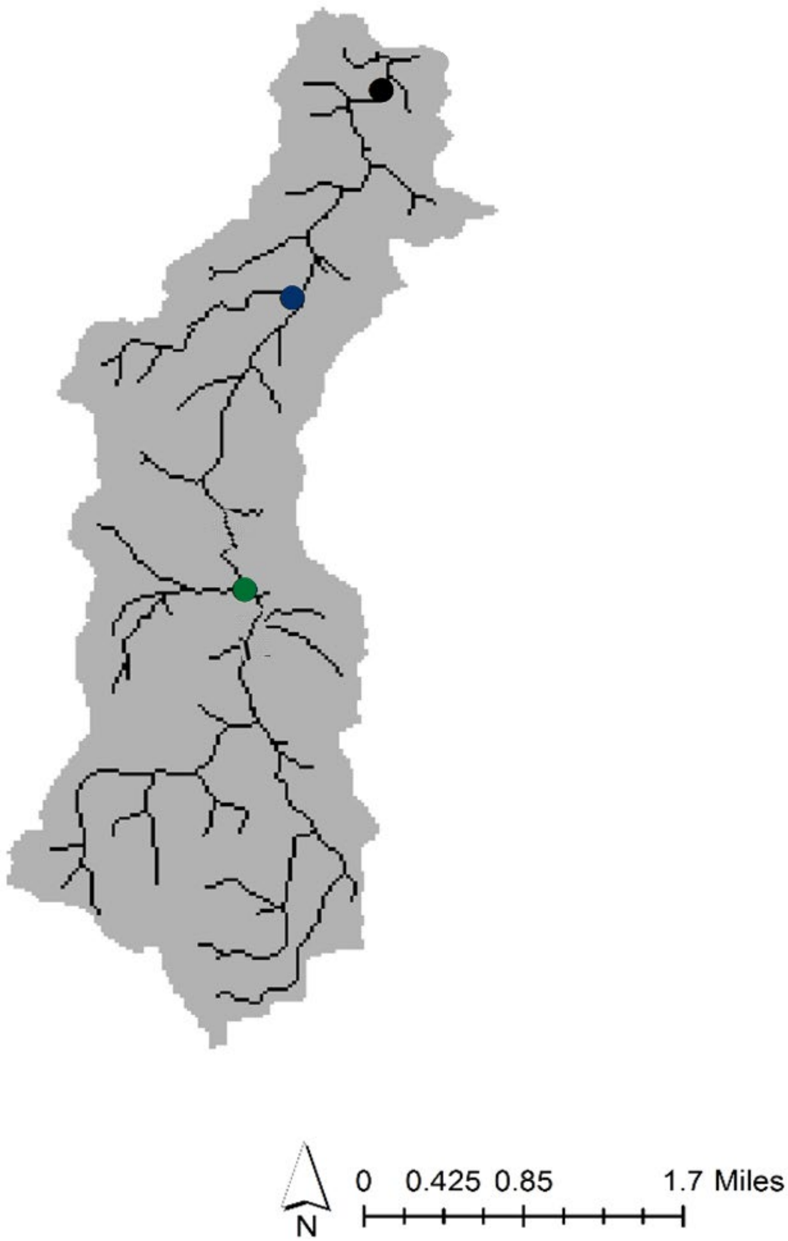

Fig. 2.1 – Río Sabana Watershed. Green circle represents low urban reach, Blue circle represent medium urban reach and Black circle represents high urban reach.

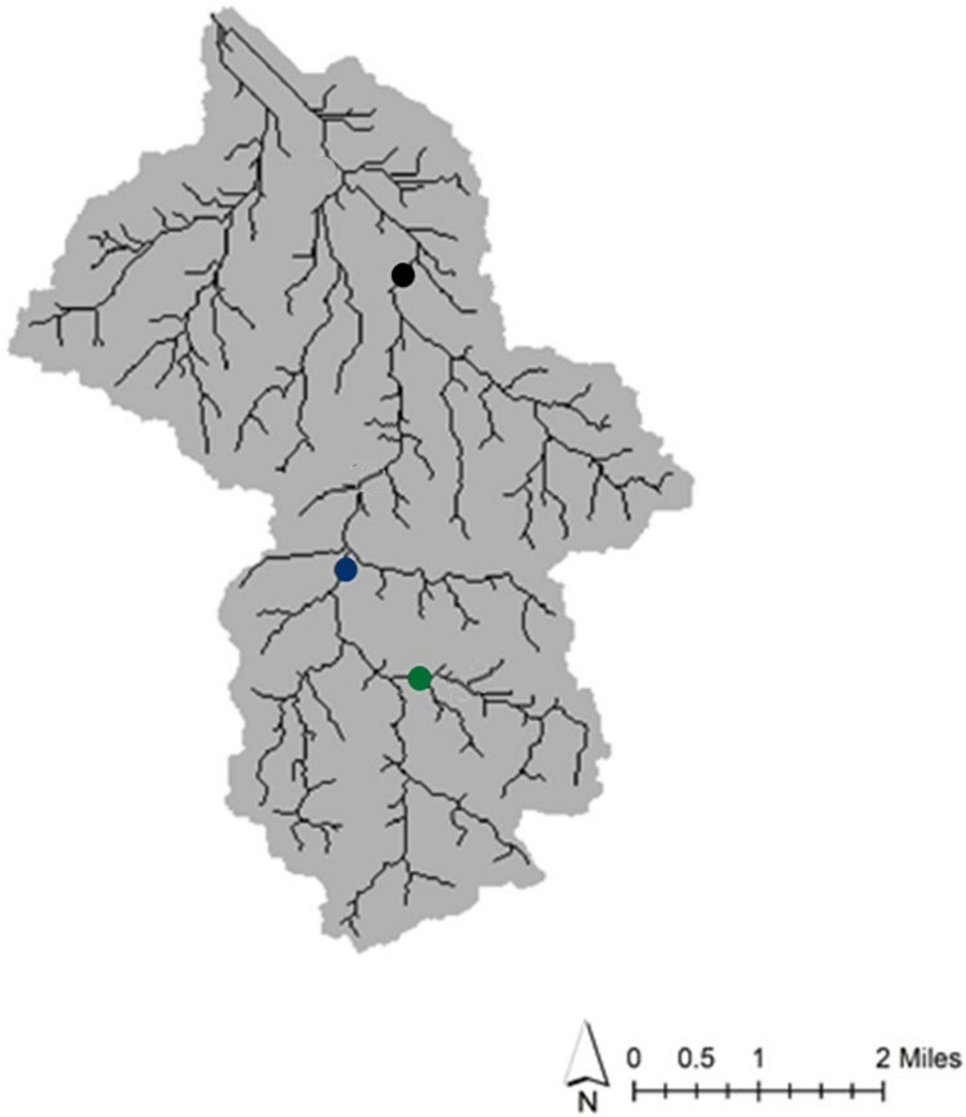

Fig. 2.2 – Río Piedras Watershed. Green circle represents low urban reach, Blue circle represents medium urban reach and Black circle represents high urban reach.

Table 2.1 – Classification of intensity of urban population in study sites reaches along the Río Piedras and Río Sabana watersheds.

Watershed	Total urban population along reach areas		
	Low	Medium	High
Río Sabana	877	1,821	5,327
Río Piedras	985	2,515	30,118

These sampling sites were classified under the following criteria: High urban (HU) - a total population between 2,500 to 50,000; Medium urban (MU) - a population between 2,500 to 1,000, and Low urban (LU) less than 1,000 inhabitants (US Census Bureau, 2012).

Table 2.2 – Mean (±SE) physicochemical measurements in low, medium and high urban reaches in Río Sabana watershed. Measurements were made during October 2018 – September 2019.

Physicochemical variables	Low urban reach	Medium urban reach	High urban reach	ANOVA
Temperature (ºC)	21.3±0.03	21.7±0.06	22.3±0.03	***
pH	7.4±0.03	7.6±0.03	7.6±0.03	***
Turbidity (NTU)	0.1±0.0004	0.1±0.0004	0.1±0.001	***
Conductivity ($\mu S.cm^{-1}$)	124±1	154±1	155±1	***
Dissolved oxygen ($mg. L^{-1}$)	8.9±0.1	9.0±0.1	0.1±0.0004	NS

NS- not significant at $p = 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 2.3 – Mean (±SE) physicochemical measurements in low, moderate and high urban reaches in Río Piedras watershed. Measurements were made during October 2018 – September 2019.

Physicochemical variables	Low urban reach	Medium urban reach	High urban reach	ANOVA
Temperature (ºC)	22.4±0.04	23.8±0.03	23.9±0.03	***
pH	7.9±0.03	7.9±0.02	8.0±0.03	***
Turbidity (NTU)	0.2±0.001	0.2±0.001	0.2±0.001	***
Conductivity ($\mu S.cm^{-1}$)	330±1	337±2	355±1	***
Dissolved oxygen ($mg.\ L^{-1}$)	7.9±0.1	7.8±0.1	7.7±0.1	***

NS- not significant at p = 0.05; * p < 0.05; ** p < 0.01; *** p < 0.001

Table 2.4 – Mean (±SE) physicochemical measurements in low, medium and high urban reaches in Río Piedras and Río Sabana watershed. Measurements were made during October 2018 – September 2019.

Physicochemical variables	Low urban reach		Medium urban reach		High urban reach		ANOVA
	Río Piedras	Río Sabana	Río Piedras	Río Sabana	Río Piedras	Río Sabana	
Temperature (°C)	22.4±0.04	21.3±0.03	23.8±0.03	21.7±0.06	23.9±0.03	22.3±0.03	***
pH	7.8±0.03	7.4±0.03	7.9±0.02	7.6±0.03	7.9±0.03	7.6±0.03	***
Turbidity (NTU)	0.2±0.001	0.1±0.0004	0.2±0.001	0.1±0.0004	0.2±0.001	0.1±0.001	***
Conductivity ($\mu S.cm^{-1}$)	330±1	124±1	337±2	154±1	355±1	155±1	***
Dissolved oxygen ($mg. L^{-1}$)	7.9±0.1	8.9±0.1	7.8±0.1	9.0±0.1	7.7±0.1	9.1±0.1	***

NS- not significant at $p = 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 2.5 – Mean (±SE) physical measurements in low, moderate and high reaches in Río Sabana watershed. Measurements were made during October 2018 – September 2019.

Physical variables	Low urban reach	Medium urban reach	High urban reach	ANOVA
Depth (m)	0.36±0.006	0.37±0.004	0.40±0.003	***
Substrate index (SI)	3.29±0.13	1.79±0.07	1.79±0.05	***
Pool canopy cover (%)	39.69±0.86	34.01±1.07	22.41±1.18	***

NS- not significant at $p = 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 2.6 – Mean (±SE) physicochemical measurements in low, moderate and high urban reaches in Río Piedras watershed. Measurements were made during October 2018 – September 2019.

Physical variables	Low urban reach	Medium urban reach	High urban reach	ANOVA
Depth (m)	0.42±0.004	0.30±0.003	0.53±0.050	***
Substrate index (SI)	1.52±0.10	1.20±0.03	1.19±0.02	***
Pool canopy cover (%)	0.53±0.050	1.19±0.02	19.21±0.67	***

NS- not significant at $p = 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 2.7 – Mean (±SE) physical measurements in low, moderate and high urban reaches in Río Piedras and Río Sabana watershed. Measurements were made during October 2018 – September 2019.

Physicochemical variables	Low urban reach		Medium urban reach		High urban reach		ANOVA
	Río Piedras	Río Sabana	Río Piedras	Río Sabana	Río Piedras	Río Sabana	
Depth (m)	0.42±0.004	0.36±0.006	0.30±0.003	0.37±0.004	0.53±0.05	0.40±0.003	***
Substrate index (SI)	1.52±0.1	3.29±0.13	1.20±0.03	1.79±0.069	1.19±0.02	1.79±0.05	***
Pool canopy cover (%)	29.53±0.99	39.69±0.86	27.33±0.99	34.01±1.07	19.21±0.67	22.41±1.18	***

NS- not significant at $p = 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

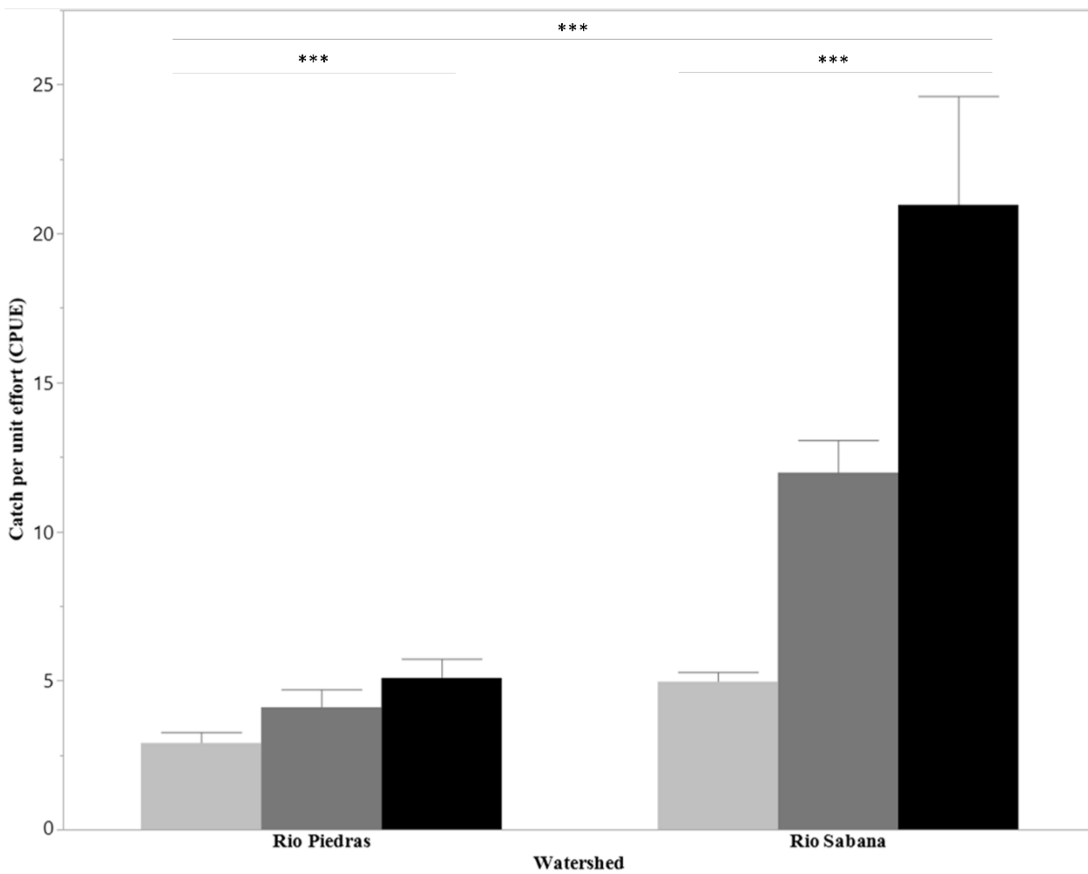

Fig 2.3 –Mean (±SE) catch per unit effort (CPUE) for *Xiphocaris elongata* in Río Piedras and Río Sabana watershed. Light grey bars represent high urban reach, grey bars represent medium urban reach and black bars represent low urban reach. Horizontal line over the bars represents the two-way ANOVA test differences NS- not significant; * p< 0.05; ** p<0.01; *** p< 0.001.

Table 2.8 – Results of Two-way analyses of variance (ANOVAs) of watersheds (Río Piedras and Río Sabana) and urban reaches (Low, Medium, High).

Source	df	F	p
Watershed	1	42.8	<0.001***
Urban reaches	2	15.9	<0.001***
Watershed X Urban reaches	2	9.3	<0.01**

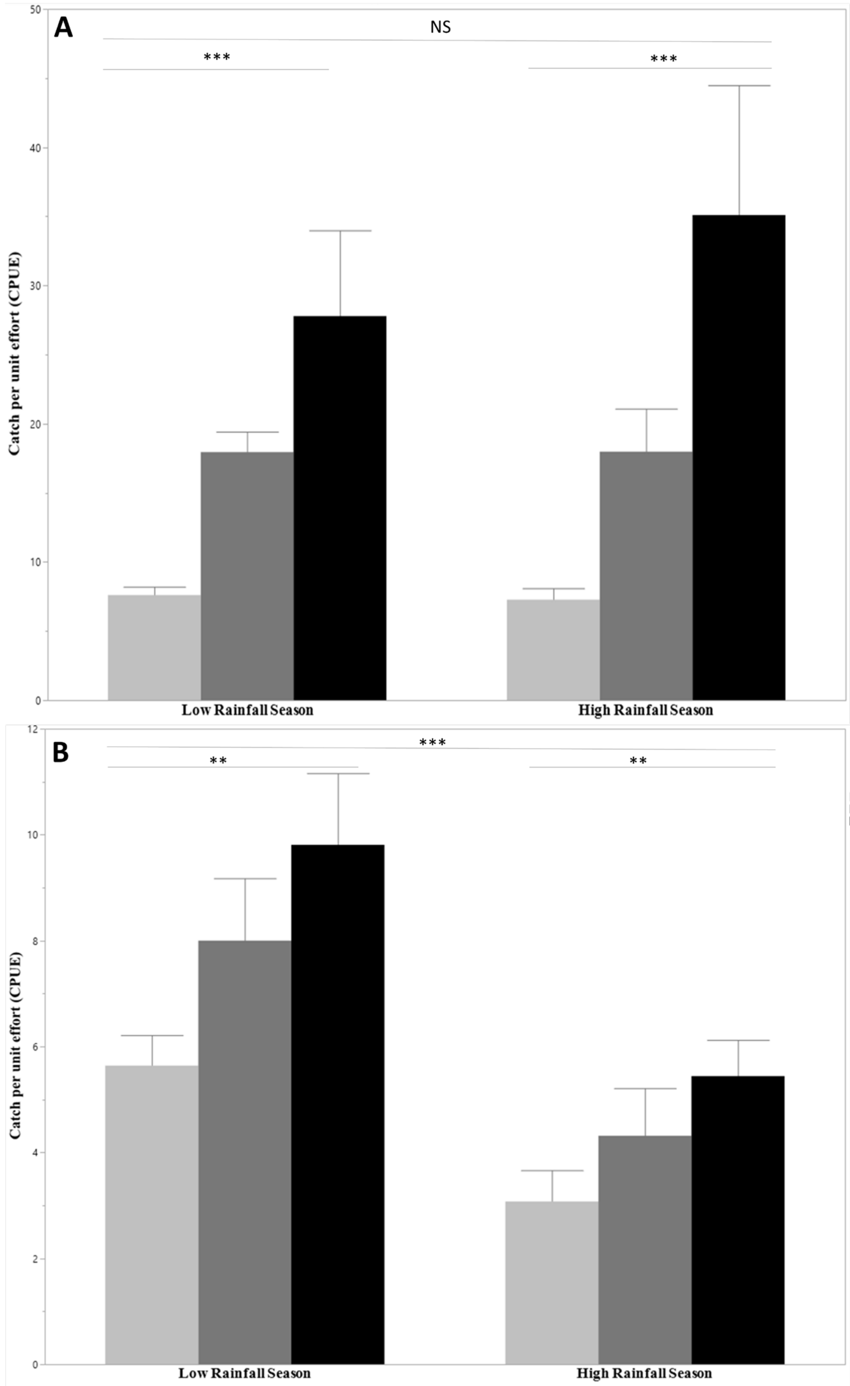

Fig. 2.4 – Mean (±SE) catch per unit effort (CPUE) for *Xiphocaris elongata* during low rainfall season and high rainfall season. A) Río Sabana and B) Río Piedras Light grey bars represent high urban reach, grey bars represent medium urban reach and black bars represent low urban reach. Horizontal line over the bars represents the two-way ANOVA test differences NS- not significant; * p< 0.05; ** p<0.01; *** p< 0.001.

Table 2.9 – Results of Two-way analyses of variance (ANOVAs) of Río Sabana urban reaches.

Source	df	*F*	*p*
Urban reaches	2	12.4	<0.001***
Rainfall seasons	1	0.4	0.6
Urban reaches X Rainfall seasons	2	0.4	0.7

Table 2.10 – Results of Two-way analyses of variance (ANOVAs) of Río Piedras urban reaches.

Source	df	*F*	*p*
Urban reaches	2	6.2	<0.01**
Rainfall seasons	1	21.9	<0.001***
Urban reaches X Rainfall seasons	2	0.5	0.62

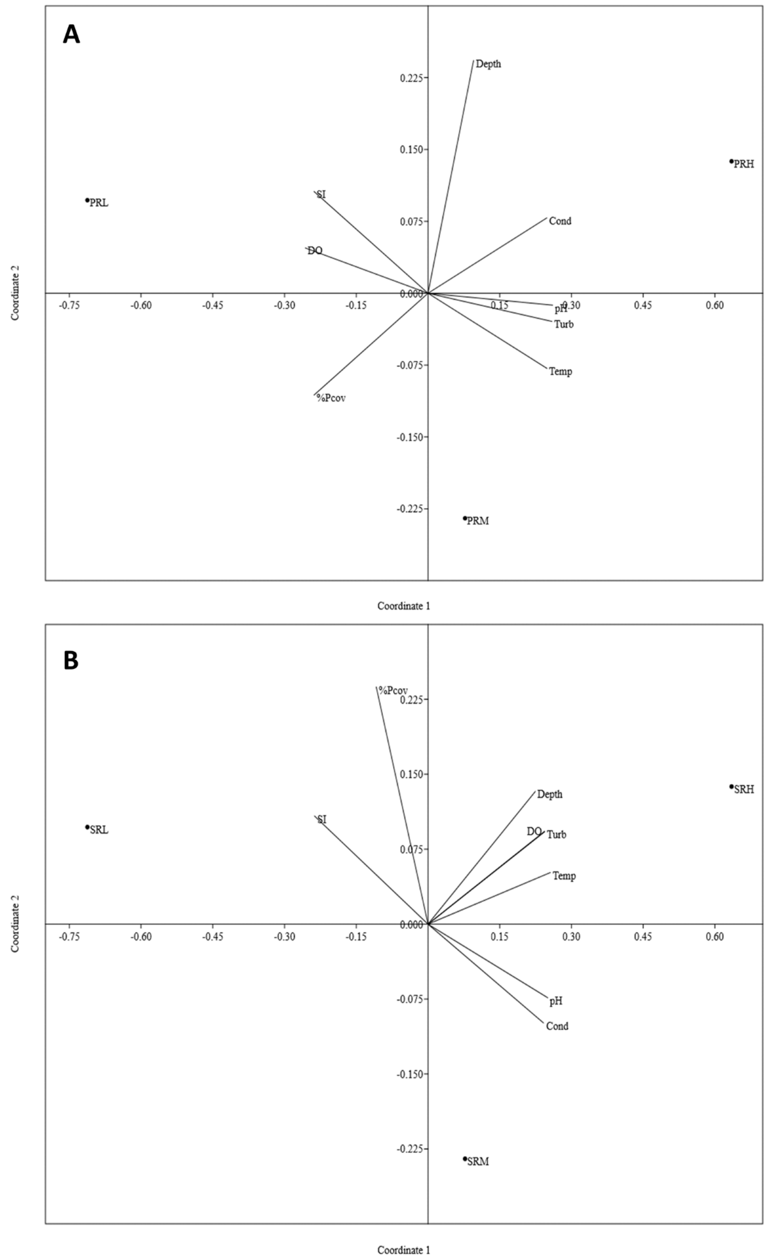

Fig. 2.5 – Nonmetric multidimensional scaling (NMDS) results from ordination of *Xiphocaris elongata* densities. Stream habitat variables measured at the different sampling sites in (A) Río Piedras (PRH – High urban reach, PRM – Medium urban reach, and PRL –Low urban reach) and (B) Río Sabana (SRH – High urban reach, SRM – Medium urban reach, and SRL –Low urban reach) were plotted as vectors. Vector length and direction reflects the strength and direction of relationship between the stream habitat variables (Depth- pool depth, SI – substrate index, Pcov- %pool cover, DO – dissolved oxygen, Cond – conductivity, Temp – temperature, Turb – turbidity) and *Xiphocaris elongata* densities.

CHAPTER III

ACUTE TOXICITY OF MALATHION, PERMETHRIN AND GLYPHOSATE ON THE TROPICAL FRESHWATER SHRIMP *XIPHOCARIS ELONGATA*

Abstract

Urban and agricultural runoffs can transport contaminants and pesticides into freshwater environment, particularly in the developing tropics. For instance, organophosphate and pyrethroids pesticides like glyphosate, malathion, and permethrin have been found in tropical streams. The uncontrolled application of these pesticides has become a growing concern due to their adverse effects on a variety of non-targeted organisms. The majority of studies have focus on a few selected model species, ignoring the effects on other non-target organisms which may play an important role in tropical lotic ecosystems. The biological characteristics of aquatic crustaceans, including their morphology, physiology and behavior, make them susceptible to toxic chemicals. For this reason, this study used the widely distributed freshwater shrimp *Xiphocaris elongata* as model organism to determinate the acute toxicity for permethrin, malathion and glyphosate. Our results show that the proportion of mortality of *X. elongata* in each concentration group became progressively higher as the concentration of exposure increased. We also found that the synthetic pyrethroid permethrin is the most toxic pesticide tested followed by the organophosphate malathion and glyphosate. Experiments with this freshwater shrimp showed a good control performance, and reproducibility for the different pesticide tested. This study demonstrated that *X. elongata* is a suitable test organism, which can act as a representative bioindicator of pesticide toxicity in tropical streams.

Keywords: *Aquatic toxicology, organophosphate, pesticide, Puerto Rico, pyrethroids*

Introduction

Pesticide contamination has become a growing concern in the tropics due to their adverse effects on a variety of non-targeted organisms. Faunal assemblages of lotic ecosystems are threatened by anthropogenic activities including pesticide pollution (Schäfer et al. 2007; Liess et al. 2005). Urban and agricultural runoff can transport contaminants and pesticides into streams (Schiff & Sutula, 2004; Zhang et al. 2010). Pesticides, however, are expected to have a much higher effect on aquatic environments because these are the ultimate recipients of contaminants (Kolpin et al. 2004; Willis & McDowell,1982). The adverse effects of pesticides can vary depending the organism and the mode of action of the chemical. The majority of ecotoxicological studies have focus on a few selected model species, ignoring the effects on other non-target organisms which may play important roles in tropical lotic ecosystems (Daam & Van den Brink, 2009; Gunnarsson & Castillo, 2018).

Organophosphate and pyrethroids pesticides like glyphosate, malathion, and permethrin have been present in many tropical freshwater environments (Buttermore et al. 2018; Santiago et al. 2016). For years, glyphosate has been a common organophosphate pesticide used to control weeds in cultivated croplands. Due to its toxicity to humans, it has been recently banned in several countries (van Bruggen et al. 2018). The primary mode of action is in the shikimate pathway that links primary and secondary metabolisms (Mensah et al. 2015). Specifically, glyphosate inhibits the activity of the enzyme 5-enolpyuvylshikimate-3-phosphate synthase (EPSPS) found in plants (Stenersen, 2004). The exposure of glyphosate to non-target organisms is of great concern to aquatic toxicologist because of their extensive use, high water solubility and slow degradation rate in freshwater environments.

Permethrin is a synthetic pyrethroid used in agricultural and residential zones to control a wide range of insects. The application of permethrin is increasing because of its use in mosquito control programs (US Environmental Protection Agency, 2005). Toxicological data for permethrin are available for a variety of aquatic organisms, but there is a need of information applicable to tropical regions (Dam & Van den Brink, 2010; US Environmental Protection Agency, 2005). Synthetic pyrethroids induce a continuous series of nerve impulses which disrupts the normal functioning of the nervous system. The mode of action is related to the inhibition of sodium channels in nerve membranes and acetylcholinesterase enzyme activity (Oliveira et al. 2012; Cox, 1998).

Malathion is a common organophosphate insecticide used to control mosquitoes and fruit flies. As an organophosphate insecticide, malathion inhibits acetylcholinesterase (AChE). Acetylcholinesterase is a critical enzyme for the normal function of the nervous system (Lionetto et al. 2011). The inhibition of this enzyme can result in the accumulation of the neurotransmitter acetylcholine in the synaptic gap leading to disruption of the nervous system (Cox, 1998). Previous studies found that malathion is highly toxic to many fish and aquatic invertebrates (Bihel & Callaghan, 2004; Printes & Callaghan, 2004; Kumar & Jawahar, 2013). The morphology, physiology, and behavior of aquatic invertebrates such as crustaceans, make them particularly susceptible to toxic chemicals (Rinderhagen et al. 2000). Because of those biological characteristics, this study used the widely distributed tropical freshwater shrimp *Xiphocaris elongata* (Guérin-Méneville,

1856) as model organism to determinate the acute toxicity of permethrin, malathion and glyphosate.

Materials and Methods

Collection and acclimation of freshwater shrimp

Adults of *Xiphocaris elongata* were collected at Río Sabana near Sabana Field Research Station in Luquillo, Puerto Rico. Organisms were captured using an electrofishing backpack (Model 12- B, Smith-Root, Vancouver, Washington, USA) and were transported alive to the laboratory. Acclimation was performed where the organisms were maintained for at least one week in aerated water, at 20°C and with a photoperiod of 12h:12h (light: dark).

Acute toxicity tests

Acute toxicity tests were performed to determine the effect of pesticides on survival of *X. elongata*. Toxicities were determinate applying a static procedure, without renewal of test solutions. All experiments were conducted in glass aquaria with a 5L capacity to which the pesticides were added at different concentrations. Toxicities were expressed based on concentration of the active ingredient rather than the formulation. Ten test concentrations and controls were used for each bioassay. Adult freshwater shrimp were selected from the acclimation tanks and distributed randomly into exposure tanks. One hundred shrimp were used for each concentration group. The organisms were not fed during the test period. After pesticide exposure, mortality was recorded between 24h and 96h.

Statistical analyses

Lethal concentration (LC_{50}) estimates were determinate using probit analyses. Dose – response results were modeled in JMP (SAS Institute, 2019). The mortality of individuals per dose were used in the probit analyses to predict the median lethal concentrations. One-way analysis of variance (ANOVA) were used to compare the mortalities rates among different test groups. A post hoc Tukey's tests was used to identify organophosphate and pyrethroids pesticides test with highly significant differences ($p<0.05$).

Results

Acute toxicity tests

The proportion of mortality of *Xiphocaris elongata* in each concentration group became progressively higher as the concentration of exposure increased. The mortality observed in acute toxicity tests for permethrin (ANOVA, $F_{9,39}=66.13$; $p <0.001$), malathion (ANOVA, $F_{9,39}=84.35$; $p <0.001$) and glyphosate (ANOVA, $F_{9,39}=107.64$; $p <0.001$) were statistically different (Table 1). Median lethal concentration (LC_{50}) values for 24h and 96h for permethrin were $6.91\text{x}10^{-6}$ µg/L and $3.96\ \text{x}10^{-6}$ µg/L, respectively (Table 3.1; Fig. 3.1 – 3.2). However, malathion median lethal concentration values for 24 h and 96 h were 13.44 µg/L and 8.87 µg/L, respectively (Table 1; Fig. 3.3 – 3.4). The least toxic pesticide was glyphosate with a LC_{50} value of 1156.4 µg/L and 748.92 µg/L for 24h and 96h, respectively (Table 1; Fig. 3.5 –3.6).

Discussion and conclusions

In this study, the acute toxicity of permethrin, glyphosate, and malathion were assessed for the tropical freshwater shrimp *Xiphocaris elongata*. To evaluate the biological outcome of such exposure, it is necessary to determinate lethal and sublethal concentrations for organisms. Due to high costs and long duration of chronic tests, there is a need to do more acute toxicity research. One advantage of using acute toxicity data is that it has been proven to be highly accurate in making long-term predictions (Kumar et al. 2010). Permethrin was the most toxic pesticide tested for our model organism followed by malathion and glyphosate. Similarly, Sánchez-Fortun and Barahona (2005) have found that the aquatic invertebrates most sensitive to pyrethroids pesticides are surface-dwelling insects, mayfly nymphs and crustaceans. Among crustacean species, *Daphnia magna*, *Daphnia pulex*, *Palaemonetes pugio* and *Macrobrachium rosenbergii*, have been used as model organisms in aquatic toxicology (Altshuler et al. 2011; Seda & Petrusek, 2011; Satapornvanit et al. 2009). However, the disadvantage of using those species is that some of them are not present in tropical freshwater environments. In tropical regions, aquatic organisms are often exposed to pesticides and other contaminants, for this reason there is a need to develop model organisms for this region such as we have done with the tropical freshwater shrimp *Xiphocaris elongata.*

Shrimp are highly susceptible to pyrethroids pesticides (Hook et al. 2018; Smith & Stratton, 1986). Even at sublethal concentrations, there could be significant behavioral changes in shrimp feeding behavior and locomotion activities that affect their survival. In this study, permethrin median lethal concentration (LC_{50}) value for 96h exposure was 6.91×10^{-6} μg/L. A study about the effect of permethrin on the grass shrimp *Palaemonetes pugio* proved to be acutely toxic at 0.21 μg/L (De Lorenzo et al. 2006). Another study found that the freshwater prawn *Palaemonetes argentinus* are very susceptible to pyrethroids with median lethal concentration of 0.0031μg/L (Collins & Cappello, 2006).

This study also determinate that malathion is high to moderate toxic with a 96h LC_{50} value of 13.44 μg/L. Similar results found a study were malathion exhibited severe toxicity to the grass shrimp *Palaemonetes pugio* with a LC_{50} of 38.19 μg/L (Key et al. 1998).

Few studies have evaluated the toxicity of glyphosate on freshwater organisms (Hong et al. 2018; Fiorino et al. 2018; Uren et al. 2014). In this study, glyphosate median lethal concentration value for 96 h was 748.92 μg/L. Mensah et al. (2011) also investigated the acute toxicity of glyphosate on freshwater shrimp *Caridina nilotica*. Their results show a median lethal concentration of 25.3 mg/L. The low toxicity of glyphosate exposure may be because shikimic acid pathway is absent in animals. However, some studies demonstrated that high concentrations could alter mitochondrial activity in animals by uncoupling of oxidative phosphorylation during cellular respiration (Mensah et al. 2015).

In summary, the tropical shrimp *Xiphocaria elongata* are an ecologically important organism in freshwater environments that can be exposed to synthetic pyrethroid and organophosphate pesticide. This study demonstrates that *X. elongata* is a suitable test or model organism, which can act as a representative bioindicator of pesticide toxicity in tropical freshwater environments. Experiments with this freshwater shrimp species showed a good control performance, and reproducibility for the different pesticides tested. Future studies should evaluate sublethal effects using environmentally relevant concentrations to specific pesticides and mixtures.

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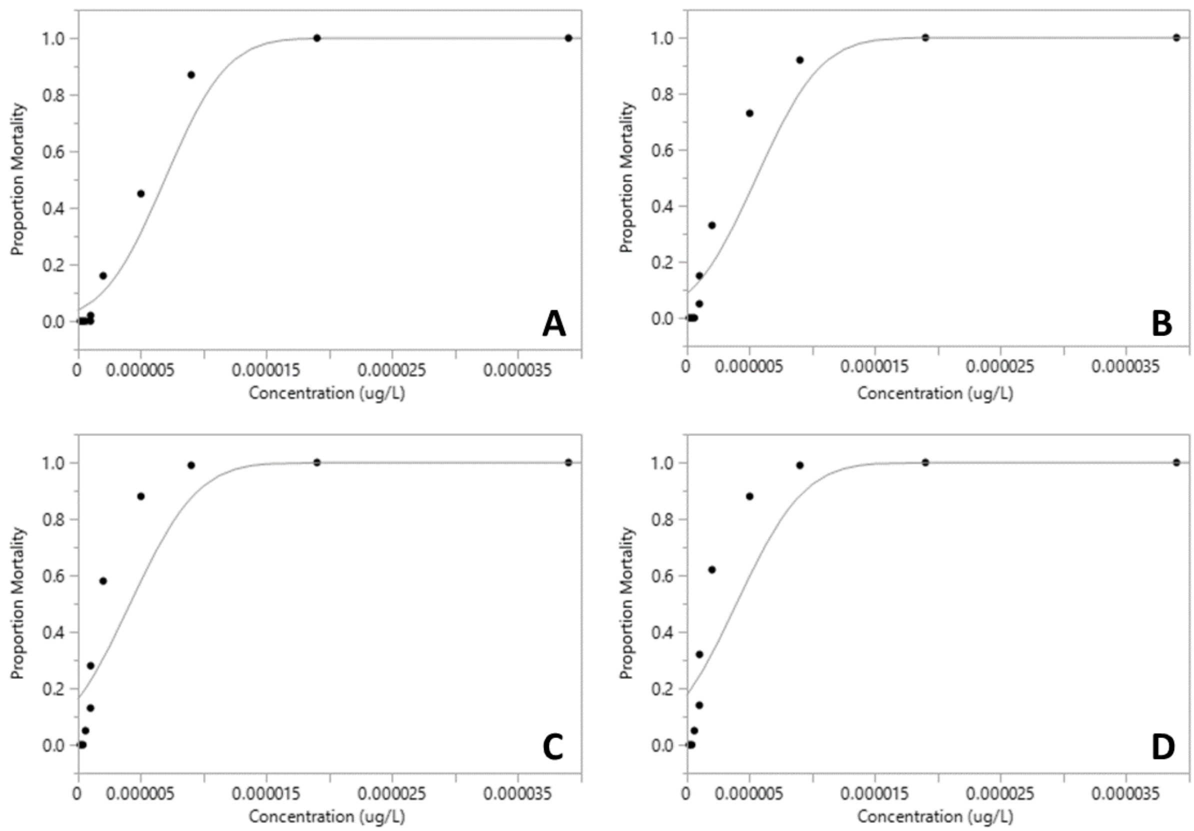

Fig 3.1 – Dose-response curves of permethrin for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

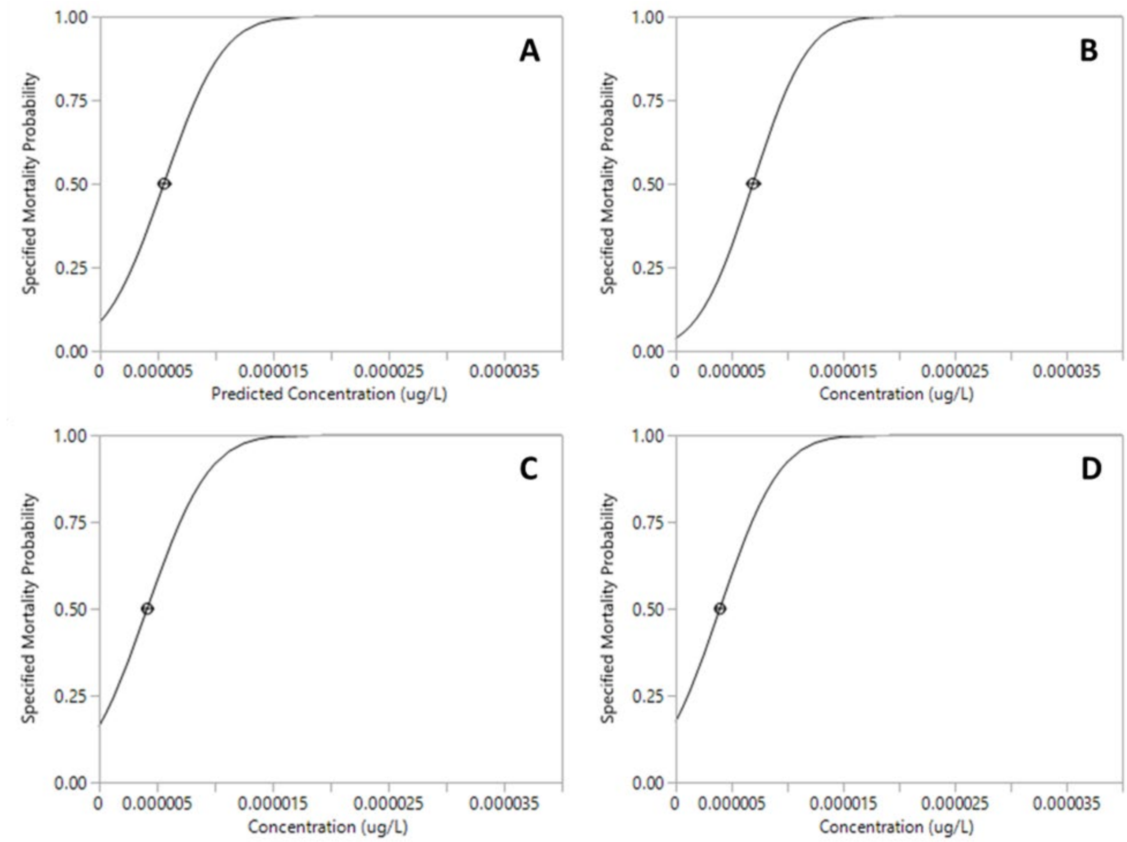

Fig 3.2 – Median lethal concentration (LC_{50}) curves of permethrin for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

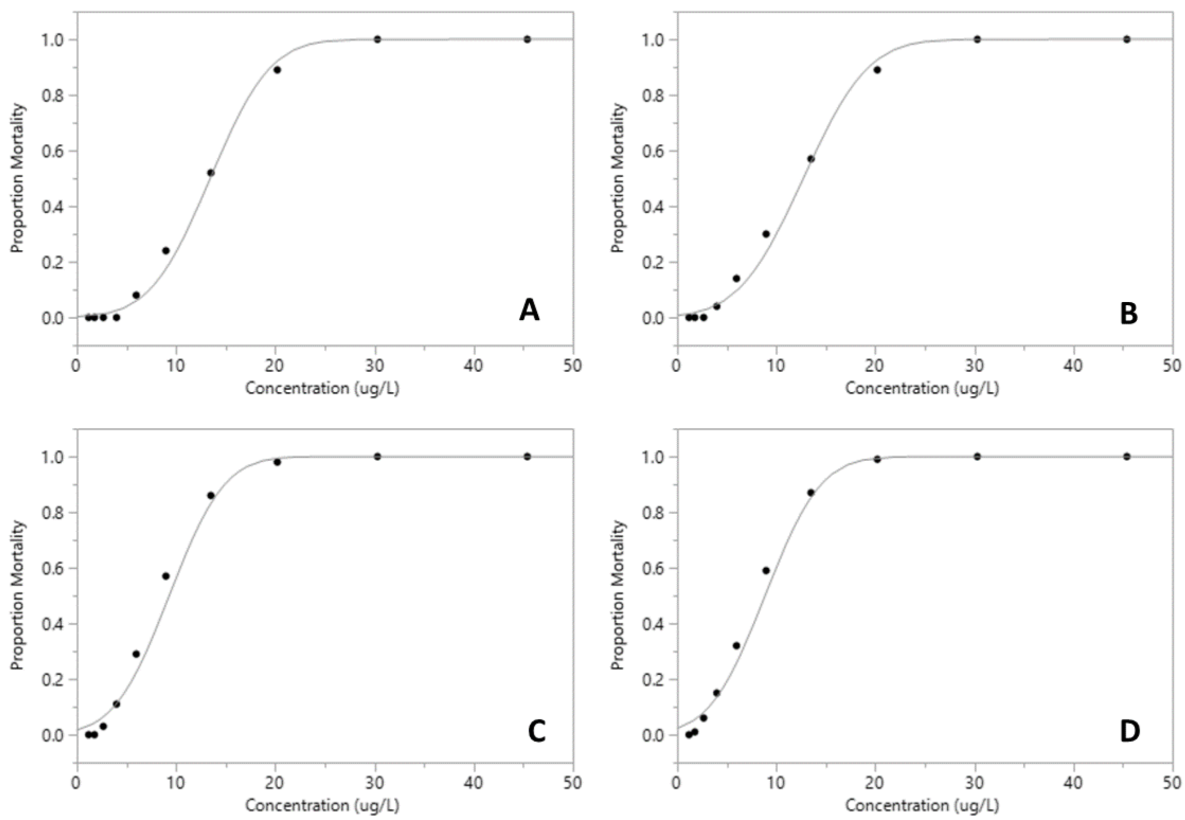

Fig 3.3 – Dose-response curves of malathion for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

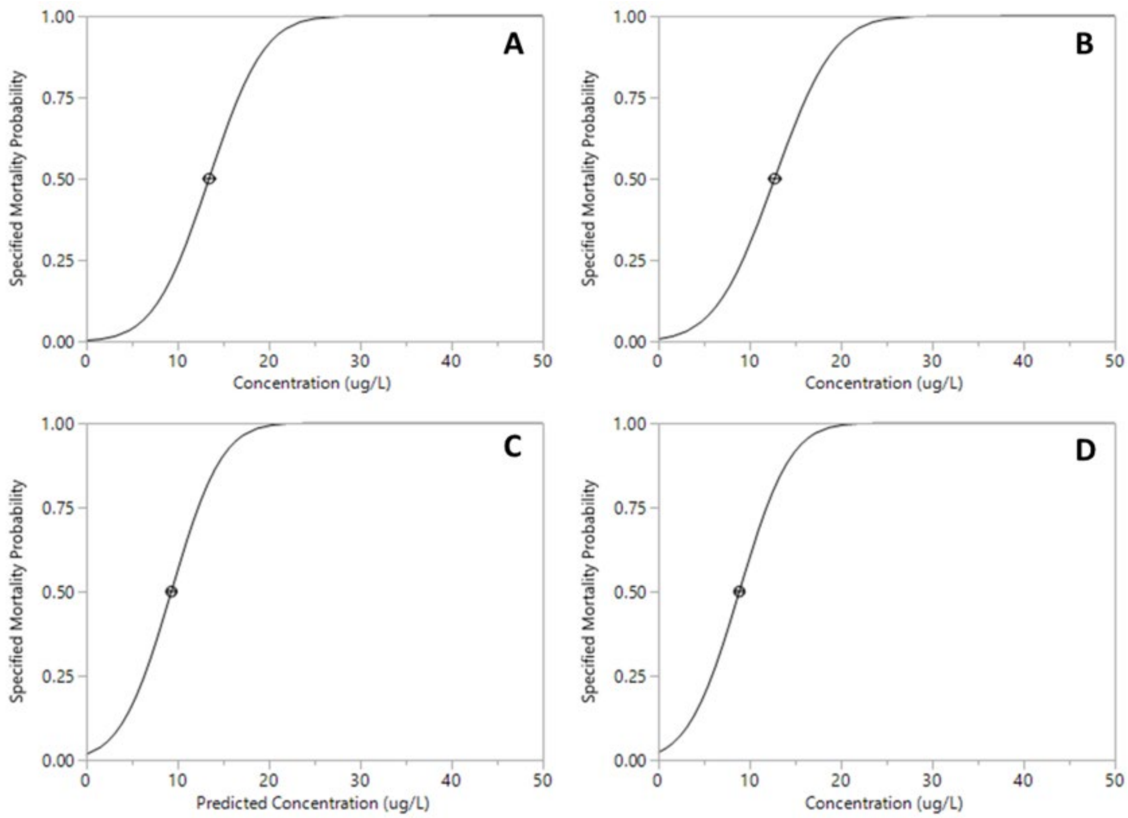

Fig. 3.4 – Median lethal concentration (LC_{50}) curves of malathion for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

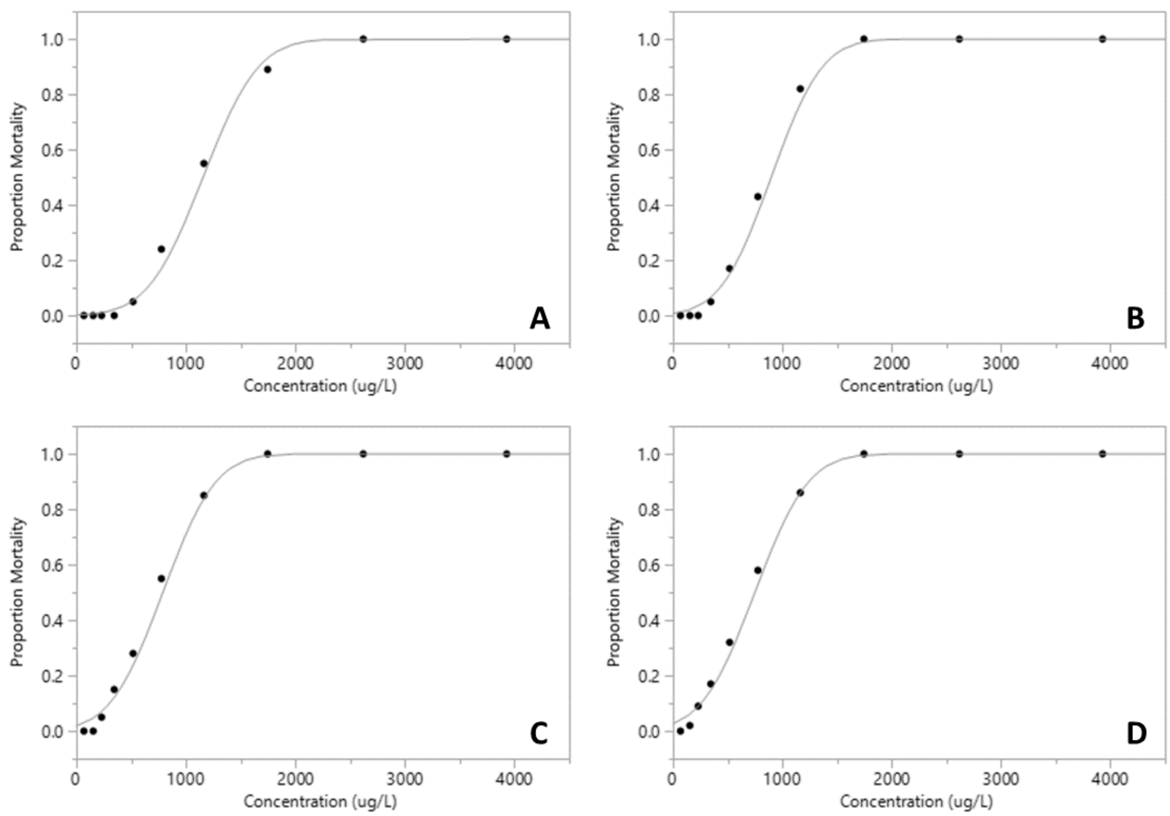

Fig. 3.5 –Dose-response curves of glyphosate for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

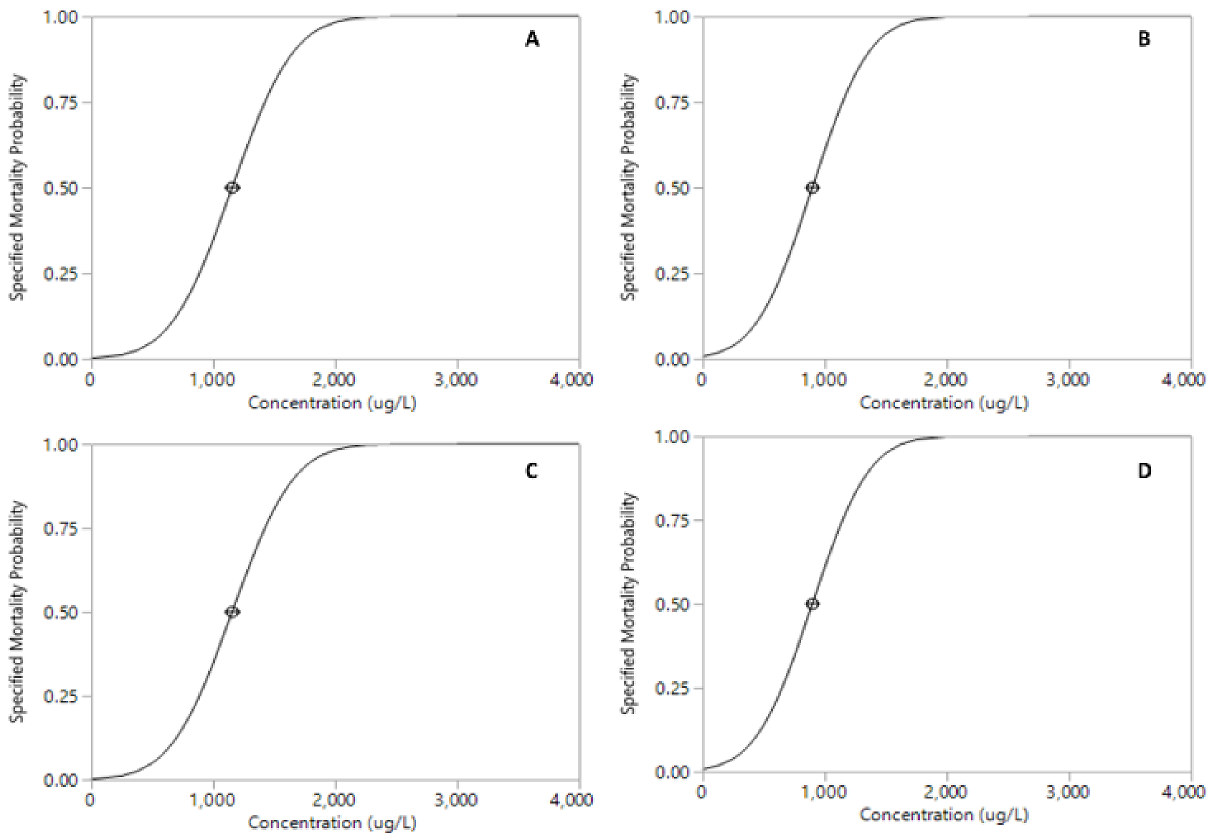

Fig 3.6 – Median lethal concentration (LC_{50}) curves of glyphosate for (A) 24 h, (B) 48 h, (C) 72 h, and (D) 96 h.

Table 3.1 – Median lethal concentration (LC_{50}) estimates for permethrin, malathion and glyphosate after 96h exposure. CI: confidence interval. Asterisks indicates significant difference * p< 0.05; ** p<0.01; *** p< 0.001.

Pesticide	LC_{50} µg /L (95% CI)	df	*F*	*p*
Permethrin	3.96×10^{-6} (4.49×10^{-6} – 4.52×10^{-6})	9	66.13	<0.001 ***
Malathion	8.87 (8.31 – 9.49)	9	84.35	<0.001 ***
Glyphosate	748.92 (701.01 – 802.45)	9	107.64	<0.001 ***

CHAPTER IV

EFFECT OF PARTICLE SIZE AND PESTICIDE CONTAMINATION ON PREFERENCE AND INGESTION RATES BY THE TROPICAL FRESHWATER SHRIMP *XIPHOCARIS ELONGATA*

Abstract

In tropical streams, freshwater shrimp are essential to preserve the structure and function of lotic ecosystems. Shredder shrimp plays a fundamental role in organic matter decomposition because they feed on detritus. In addition, shredders shrimp are very important organisms as they connect all functional groups of food webs. In natural environments, decaying leaf material may accumulate contaminants, including insecticides and herbicides. Thus, shredder shrimp can be exposed to contaminants through ingestion of leaf litter material. The objectives of this study were to evaluate if the shredder shrimp *Xiphocaris elongata* display preference for feeding on different leaf species and leaf size area, while also assessing their consumption of leaves contaminated with pesticides. We also evaluated acetylcholinesterase (AChE) activity as a possible biomarker of pesticide contamination using an immunofluorescence and microscopy imaging approach. Our results revealed that the leaf area and plant species more appropriate for future toxicological studies is *Spathodea campanulata* leaves with a leaf area of 0.65 cm^2. This study also showed that sublethal concentrations of malathion and permethrin in leaves seems to have a significant effect on ingestion rates of *X. elongata*, which suggests that the presence of these contaminants had an influence on feeding behavior. Immunofluorescence in cephalothorax ganglia showed a decline in AChE activity when sublethal dose of malathion and permethrin increases. The observed results suggest that AChE activity can

be used as biomarker for the detection and assessment of permethrin and malathion exposure on shredder shrimp.

Keywords*: Malathion, Permethrin, Pesticide, Puerto Rico, Toxicology*

Introduction

Freshwater shrimp are essential to preserve the structure and function of tropical lotic ecosystems. These organisms have important role as biological indicators of stream health, and much is known about their ecology in the tropical island of Puerto Rico (Covich et al. 1991; 1996; 2009). A total of 17 species from three families have been described for the island (Pérez-Reyes et al. 2013). Among the families, Xiphocarididae plays a fundamental role in organic matter decomposition because these organisms feed on senesced plant parts such as leaves, and detritus. They can also serve as prey to various terrestrial and freshwater organisms like wading birds (Miranda & Collazo, 1997) and diadromous predatory fishes (Covich et al. 2009; Ocasio-Torres et al. 2014). Thus, shredder organisms are very important as they connect functional groups of stream food webs. However, few toxicological studies have focused on tropical shredder species.

In this study, the shredder shrimp *Xiphocaris elongata* (Guérin-Méneville, 1856) was selected as model organism. Shredder shrimp are important processors of allochthonous organic carbon inputs and a food source for other aquatic and terrestrial organisms (Baxter et al. 2005; Covich & McDowell, 1996; Cummins et al. 1989). In natural environments, decaying leaf material may accumulate pollutants, including insecticides and herbicides present in surface water (Englert et al. 2017; Kreutzweiser et al. 2007; Rasmussen et al. 2012). Thus, shredder shrimps can be exposed to pesticides through ingestion of contaminated leaf litter material (Forrow & Maltby, 2000; Wilding & Maltby, 2006). Pesticides can be divided into four main groups: organochlorines, organophosphates, carbamates and pyrethroids. All these pesticide groups can have a deleterious effect at behavioral, physiological and biochemical levels (García-de la Parra et al. 2006; Khazri et al. 2016; Terçariol & Godinho, 2011; Tu et al. 2010), which can result in changes in composition, structure and function of the stream community (Forrow & Maltby, 2000).

The ability of shredders to feed on leaf litter selectively suggests that foraging decisions made by these organisms can be affected by contaminated leaves. In addition, exposure to contaminated leaf litter can decrease feeding rates, thus reducing growth, size, fecundity, and survival of individuals (Anderson & Cummins,1979; Harmon & Wiley, 2010; Swan & Palmer, 2006). The ability of freshwater shredder organisms to use different species of leaf litter as a food source has been evaluated in many studies (Chará-Serna et al. 2012, Bastian et al. 2007; Crowl et al. 2006). Most feeding preference studies focused on the importance of microbial colonization of leaf litter for palatability (Danger et al. 2012; Foucreau et al. 2016). However, fresh and brown unconditioned leaves can also be use by shredder organisms and are sometimes even preferred (Gessner et al. 1991; Graça & 2010; Stout et al. 1985).

Leaves are one of the entry routes of pesticides inside benthic aquatic organisms because decaying leaf material can accumulate pollutants (McKnight et al. 2015). Organophosphate and pyrethroid pesticides, like malathion and permethrin, inhibits acetylcholinesterase (AChE) activity. Acetylcholinesterase is a critical enzyme in the normal function of the nervous system. The inhibition of this enzyme can result in the accumulation of the neurotransmitter acetylcholine in the synaptic gap leading to disruption of the nervous system. The inhibition of AChE activity has been used as a biomarker in invertebrates to detect and evaluate contamination by anticholinesterase pesticides (Lionetto et al. 2011).

The objectives of this study were to evaluate if the shredder shrimp *Xiphocaris elongata* display preference for feeding on different leaf species and leaf size area, while also assessing their consumption of leaves contaminated with pesticides. No-choice assays were done to determinate which of the different leaf species and size areas the shredder shrimp would prefer when they had no alternative available. In addition, we evaluated acetylcholinesterase (AChE) activity as a possible biomarker of pesticide contamination using an immunofluorescence and microscopy imaging approach.

Materials and Methods

Collection and acclimation of organisms

Adults of *Xiphocaris elongata* were collected at Río Sabana near Sabana Field Research Station in Luquillo, Puerto Rico. Organisms were captured using an electrofishing backpack (Model 12- B, Smith-Root, Vancouver, Washington, USA) and were transported alive to the laboratory. Acclimation was performed where the organisms were maintained for at least one week in aerated water, at 20°C and with a photoperiod of 12h:12h (light: dark).

Leaves collection and preparation

Three common riparian species found in forest and urban streams in the island, the trumpet-tree *Cecropia schreberiana* (Berg et al. 1990), the African tulip tree *Spathodea campanulata* (Palisot de Beauvois, 1805), and the Guyanese pepper *Piper glabrescens* (de Candolle, 1869) were used in this study. Green leaves were picked from trees, and oven-dried at 55 ºC for 48 h. For brown leaves, leaf litter baskets were installed in the riparian forest to collect senescent leaves from trees, and oven-dried at 55 ºC for 48 h. To mimic the conditioning history of leaf litter entering the streams, green leaves were picked from trees, air dried overnight, placed in nylon mesh bags, and exposed in the stream for two weeks. At the end of the two weeks, leaves were removed from the bags, and dried at room temperature for 12 h. All leaf squares were cut in three different size categories (0.65 cm^2, 1.3 cm^2 and 2.6 cm^2), weighed, and placed in aquariums for shrimp feeding trials.

Leaf size area and species preference test

To evaluate *X. elongata* preference for riparian plant species and leaf size area that prefer, bioassays were performed where the shrimp only have one available size of pre-weighed leaf squares. Organisms were not allowed to feed 48h prior to the beginning of the test. The exposure tanks are assembled with two cameras: the upper camera, where the organism will be exposed, which has holes in the bottom surface to prevent the contact of the organism with detritus and feces that fall into the bottom of the second camera (Fig 4.1). Control tanks only had leaf squares to evaluate autogenic changes in the absence of the organism. The preference test was monitored daily for 96 h. During the test period, temperature, dissolved oxygen, turbidity, conductivity and pH were recorded. At the end of the test, the remaining leaf squares were frozen for 24 h to eliminate further breakdown, oven-dried at 55 ºC for 48 h, and weighed in order to determine the feeding rate. All shrimp were measured and weighed before and after exposure.

Leaves with pesticide contamination test

To evaluate if *Xiphocaris elongata* reveal some changes in feeding behavior between contaminated and uncontaminated leaves, bioassays with permethrin and malathion saturated leaves were separately performed. Organisms for these tests were not allowed to feed 48h prior to the beginning of the test. In experimental tanks, pre-weighed leaf squares contaminated with the pesticides were added (Fig 4.2). For each pesticide, two sublethal concentrations of permethrin (1.00×10^{-6} µg/L and 2.00×10^{-6} µg/L) and malathion (6.00 µg/L and 7.00 µg/L) were used. At the end of the experiments, leaf squares were frozen for 24 h to eliminate further breakdown, and oven-dried at 55 ºC for 48 h. Finally, they were weighed in order to determine the feeding rate. All shrimp used were measured and weighed before and after exposure. During test period, temperature, dissolved oxygen, turbidity, conductivity, and pH were recorded daily. Ingestion rates were calculated using the following formula:

$$I = \frac{\Delta Lw}{Lw_i} \cdot \frac{1}{T \cdot Shw}$$

where, ΔLw represents the changes of leaves weight in µg (Lw_i - Lw_f); Lw_i is the leaf initial weight; T is the time expressed in days, and Shw (mg) is the shrimp weight. Ingestion rate is reported in $\mu g \cdot mg^{-1} \cdot day^{-1}$.

Effects of pesticides in nervous cord: Immunofluorescence and image analysis

In total, thirty individuals of *Xiphocaris elongata* were subjected to bioassay tests. Twenty individuals were exposed to three feeding trials with leaf squares contaminated with malathion and permethrin. While, ten individuals were exposed to three feeding trials with uncontaminated leaf squares. Forty-eight hours after completing the bioassay, the individuals were anesthetized with 10 mL of a 1.09 mM solution of Linalool and were dissected to remove their nervous cords. Removed tissues were fixed in 4% paraformaldehyde. The nervous cords were divided into segments to separate the cephalothorax ganglia from abdominal ganglia.

Cephalothorax ganglia were permeabilized in HBSS/ Sucrose + 0.1% saponin solution (HBSS:Su:Sap) for 10 minutes and blocked in 5% Fetal bovine serum (FBS) diluted in HBSS:Su:Sap for 15 minutes, followed by α-Bungarotoxin, Alexa Fluor™ 555 conjugate diluted in HBSS:Su:Sap:FBS serum for 1 hour at RT. Then a neuronal stain was added NeuroTrace™ 640/660 Deep- Red Fluorescent Nissl stain (ThermoFisher Scientific/ Life Technologies, Cat. #N21483, Lot. #2047616) (1:25) diluted in HBSS for 1 h at RT, followed by the nuclear counterstain DAPI (ThermoFisher Scientific/ Molecular Probes, Cat. #D1306, Lot. #2031179) (10µM) diluted in HBSS for 1 h at RT. Images were taken on an Olympus Inverted Epifluorescence Microscope with an objective Olympus LUCPlanFLN 40x/0.60 ph2 ∞ /0-2/FN22. Image J was used to quantify AChE stained by α-bungarotoxin, we measured the number and intensity of fluorescent pixels in the images by setting the intensity of fluorescence with a control ganglia image and comparing the mean fluorescence intensity in control and pesticide exposed ganglia.

Statistical analyses

The statistical analyses were performed in JMP Pro 13 (SAS Institute, 2019). Three-way analyses of variance (ANOVAs) were used to test the effects of plant species, leaf area and exposure treatment. In addition, one-way analysis of variance (ANOVA) were used to compared total ingestion rates of contaminated versus uncontaminated leaves, and mean fluorescence intensities from the immunofluorescent experiment. A post hoc Tukey's tests was used to identify treatment groups of highly significant differences ($p<0.05$).

Results

Leaf area and plant species preference

The mean and standard error values for the physicochemical parameters measured during the leaf area and plant species preference assays were temperature 20.9±0.1°C, conductivity 349±1.80µS, pH 7.81±0.01, dissolved oxygen 9.01±0. 01mg.L^{-1} and turbidity 174±0.69 ppm. Significant differences (Three-way ANOVA, $F_{2,39}$=49.84; p <0.001) were found for the ingestion rates of different leaf sizes (Table 4.1; Fig. 4.3). Leaves with an area of 0.65cm^2 were ingested in higher quantity for all plant species. Significant differences (Three-way ANOVA, $F_{2,39}$=450.39; p <0.001) were also found for the different plant species in the preference test. *Spathodea campanulata* conditionated leaves were preferred and ingested in a higher quantity (0.49±0.03 µg•mg-^{1}• day^{-1}). Brown *Piper glabrescens* leaves were the least ingested (0.041±0.004 µg•mg-^{1}•day^{-1}) (Fig. 4.4).

Leaves with pesticide contamination test

Significant differences were observed (ANOVA, $F_{2,24}$ = 26.958; p < 0.001) for ingestion rates in the permethrin assay (Fig. 4.5). Higher concentration of permethrin had a lower ingestion rate than the control group (0.64 ±0.05 µg.mg^{-1}.day^{-1}). The ingestion rates of malathion contaminated leaves were also statically different from control group (ANOVA, $F_{2,24}$ = 24.281; p < 0.001; Fig. 4.5). Physicochemical parameters for the permethrin and malathion assays are summarized in Table 4.2.

Effects of pesticides in nervous cord: Immunofluorescence and image analysis

Control and pesticides exposed shrimp ganglia were stained with α-bungarotoxin to label acetylcholinesterase (AChE) in the cephalothorax segments (Fig. 4.6). α-

Bungarotoxin staining appeared qualitatively similar in both control and pesticide exposed ganglia. However, the mean fluorescence intensity was statistically different between control, permethrin (ANOVA, $F_{1,9} = 7.58$; $p < 0.05$) and malathion (ANOVA, $F_{1,9} = 8.45$; $p < 0.05$) exposed ganglia indicating that AChE activity could be affected by these pesticides (Fig 4.7).

Discussion

Toxicological feeding experiments with freshwater organisms should be developed with diets that provide all nutritional requirements. It is important to know what particle size area, quantity and species they should be fed. Food particles should be of a size that maximizes ingestion and minimizes waste (Azaza et al. 2010). Thus, one of the objectives of this study was to determinate if the shredder shrimp *Xiphocaris elongata* demonstrated preference for feeding on riparian plant species and leaf areas. These results contribute to establish a leaf area and species that maximizes ingestion for further toxicological feeding studies. Shredder shrimp feeds on coarse particulate organic matter (CPOM), so probably the preferences of the leaves size are expected to be different depending on plant species.

In laboratory assays, in order to minimize the number of variables, only one species and leaf size were provided. When different leaf sizes were offered, *X. elongata* presented a significant preference for smaller area, while bigger leaf areas (2.6 cm^2) resulted in lower ingestion rate for all three plant species. This study demonstrated that *Spathodea campanulata* leaves, with an area of 0.65 cm^2, present the highest ingestion rates, followed by *Cecropia schreberiana* and *Piper glabrescens* leaves. Similarly, when different leaves species were offered, *X. elongata* presented a significant preference for *S. campanulata*, followed by *C. schreberiana* and *P. glabrescens* leaves for all size areas.

Leaves are one of the entry routes of contaminants for several aquatic organisms (McKnight et al. 2015). As shredder organisms, *X. elongata* feeding on detritus are exposed to pesticide present in the contaminated plant tissue. Additionally, there is growing evidence that pesticide toxicity is an important issue in aquatic environments, especially in tropical regions (Gunnarsson & Castillo, 2018; Daam & Van den Brink, 2009). Given the fact that some types of pesticide are present in surface waters, the need to evaluate if these contaminants affect feeding behavior under different concentrations scenarios becomes urgent. Base on the results from assays where contaminated and uncontaminated leaves were offered, it was evident that the increasing concentration of permethrin and malathion on leaves has a significant effect on shrimp feeding behavior.

For permethrin, the ingestion rate of *X. elongata* decreases significantly at both lower and higher concentrations than compared to uncontaminated leaves. Therefore, it seems that the presence of this pesticide influences ingestion rates. The ingestion rate was slightly higher when leaves were spiked with a low concentration of permethrin (1.00 x 10-6 $\mu g.l^{-1}$) compared to higher concentration of the pesticide. In a previous study using a similar assay method, permethrin affected the feeding rate of the insect *Plutella xylostella*. Leaf consumption was negatively correlated with the quantity of permethrin present, which could have been due to a decrease in feeding rate after ingestion (Hoy & Hall,1993). Similarly, Armstrong and Bonner (1985) found that permethrin exposure produced a significant reduction in feeding behavior of the fruit fly *Drosophila melanogaster*.

In the malathion assay, an increase in concentration of the organophosphate pesticide in leaves seemed to have a significant effect on the ingestion rates of *X. elongata*, which suggests that the presence of this pollutant had an influence on feeding behavior. In the present study, the ingestion rate was significant lower when leaves were spiked with a high (7.0 μg.l^{-1}). and low (6.0 μg.l^{-1}) concentration of malathion. Similar results were found in a previous study using the shrimp *Macrobrachium nipponense* as model organism. They found that malathion significantly reduced feeding rates as the concentration of the pesticide increased (Yuan et al. 2004). Considering the feeding assays results for the shrimp *X. elongata*, the ingestion rates seem to be affected by the presence of leaves having high concentration of pesticides. The presence of pyrethroids and organophosphorus pesticide in leaves can influence feeding behavior. Exposure to malathion and permethrin have also shown dose–response relationships: as the pesticide concentrations increased, feeding rates decreased.

Acetylcholinesterase (AChE) activity inhibition is well known as a biomarker indicating the effect of neurotoxic pollutants (Lionetto et al. 2011). In this study, we used an immunofluorescence approach to evaluate the effect of sublethal concentrations of malathion and permethrin on AChE activity. The immunofluorescence results showed a significant difference in AChE activity between pesticide exposed ganglia and control group ganglia. AChE activity has also been found to decrease in other species of shrimp exposed to sublethal concentrations of malathion (Kumar & Jawahar, 2013). Another study found similar results that demonstrated that malathion caused reduction in *Daphnia magna* AChE activity levels by up to 50% with an adverse effect on mobility (Printes & Callaghan, 2004). For permethrin, few studies have been reported regarding the effect on AChE activity. However, Khazri et al. 2016 showed that AChE activity in gills of the mussel *Unio ravoisieri*, significantly decreased with increased concentration of permethrin. The decrease in AChE activity observed in this study could be due to the decrease of the enzyme synthesis by the inhibitory characteristic of malathion and permethrin pesticides.

Generally, a reduction of AChE activity is observed after exposition to sublethal concentrations of pyrethroids and organophosphate pesticide. For example, Jebali et al. 2006, after an administration of sublethal concentrations of malathion, observed a decrease in AChE activity, after 2 and 7 days of the treatment, compared to controls in the brain tissue of the fish *Seriola dumerilli*. Chandra (2008) showed a maximum inhibition of 77.12% and 72.83% in brain and gills, respectively after exposition of the freshwater catfish *Heteropneustes fossilis* to malathion. Moreover, Ibrahim et al. 1998 found that AChE activity in the dipterian *Chironomus riparius* significantly declines when the animals are exposed to permethrin.

Conclusion

Overall, the current results lead to the conclusion that the shrimp *Xiphocaris elongata* prefer certain leaf area and plant species when no-alternative were offered. The leaf area and plant species more appropriate for future toxicological studies are *Spathodea campanulata* leaves with an area of 0.65 cm^2. This study also showed that sublethal concentrations of malathion and permethrin in leaves seems to have a significant effect on ingestion rates of *X. elongata*, which suggests that the presence of these contaminants had an influence on feeding behavior. In addition, immunofluorescence in cephalothorax ganglia showed a decline in AChE activity when a sublethal dose of malathion and permethrin increases. The observed results suggest that AChE activity can be used as biomarker for the detection and assessment of organophosphate and pyrethroid pesticide exposure on non-target species like the common shredder shrimp *Xiphocaris elongata*.

This study highlights the importance of the abundantly distributed shredder freshwater shrimp as a potential bioindicator organism to assess the risk of pesticide contamination in freshwater environments.

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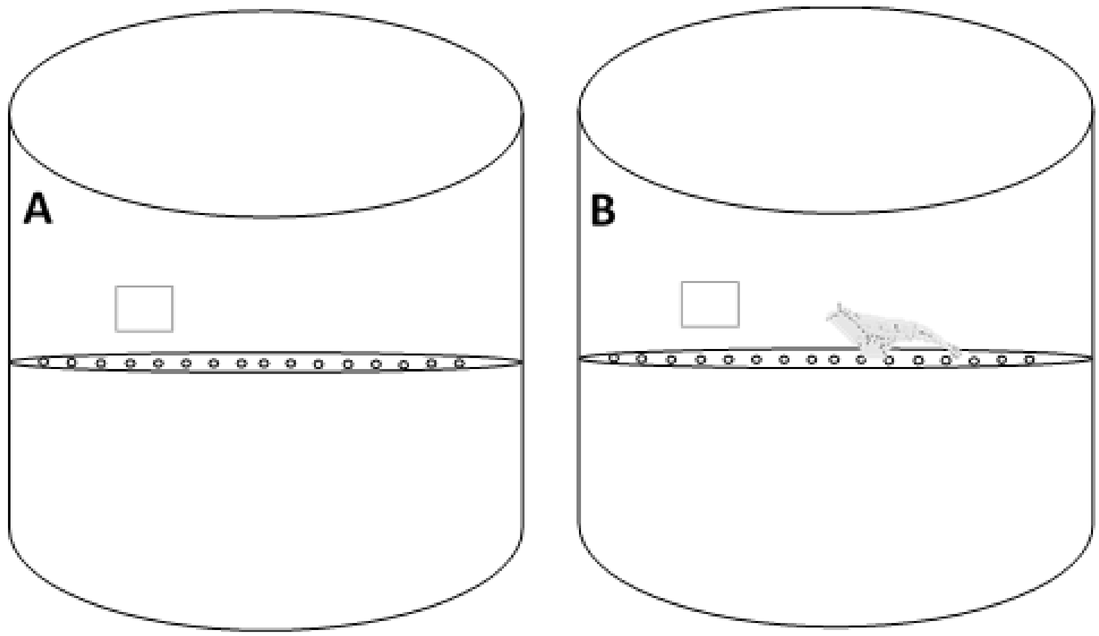

Fig 4.1 –Exposure tanks have two cameras assemble: the upper camera that have holes to prevent the contact of the organism with detritus that fall in the bottom of the second camera. A) Control tanks only had leaves square to evaluate autogenic changes in the absence of the organism. B) Experimental tanks had a leaf square with the shrimp to evaluate the ingestion rate. White square – leaf.

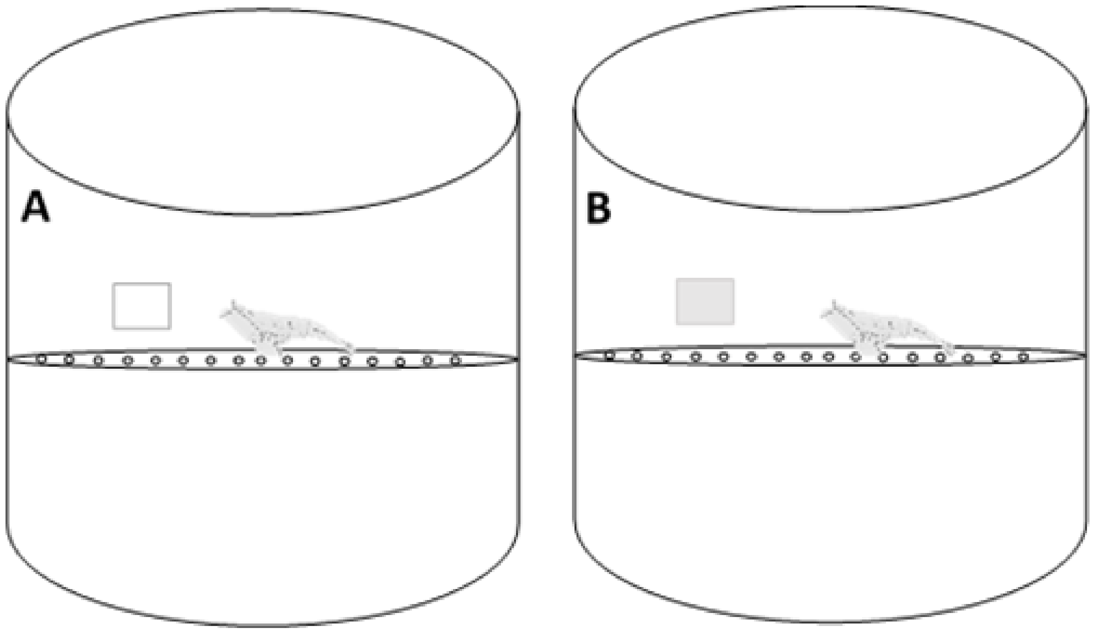

Fig 4.2 – Exposure tanks with two cameras were used to evaluate changes in ingestion rate between uncontaminated and contaminated leaves. Organisms for these tests were not allowed to feed 48h prior to the beginning of the test. A) Control tanks had an uncontaminated leaf square with the shrimp. B) Experimental tanks had a contaminated leaf square with the shrimp to evaluate changes in ingestion rate. White square – uncontaminated leaf and Grey square – contaminated leaf.

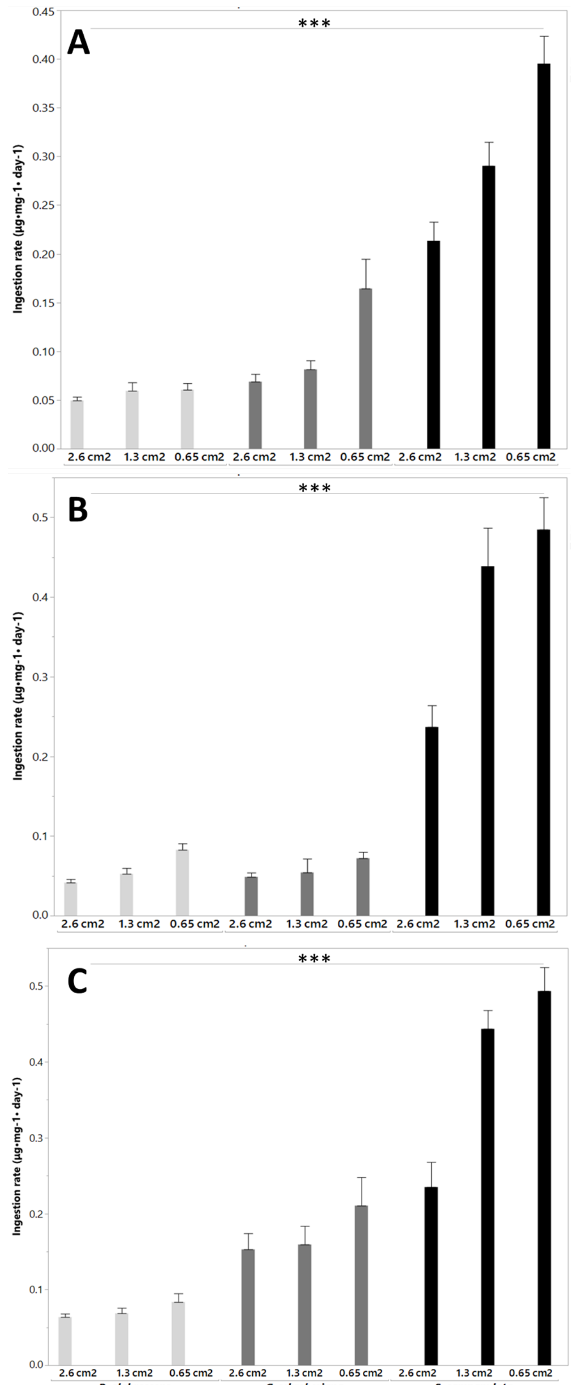

Fig. 4.3 – Mean (±SE) ingestion rate (µg•mg^{-1}• day^{-1}). A) Green leaves, B) Brown leaves, C) Conditionated leaves for each size area (0.65 cm^2, 1.3 cm^2 and 2.6 cm^2) and plant species (*S. campanulata, C. schreberiana and P. glabrescens*). White bars – *P. glabrescens*, Grey bars – *C. schreberiana*, and Black bars – *S. campanulata*. Horizontal line over the bars represents the ANOVA test differences NS- not significant; * p< 0.05; ** p<0.01; *** p< 0.001.

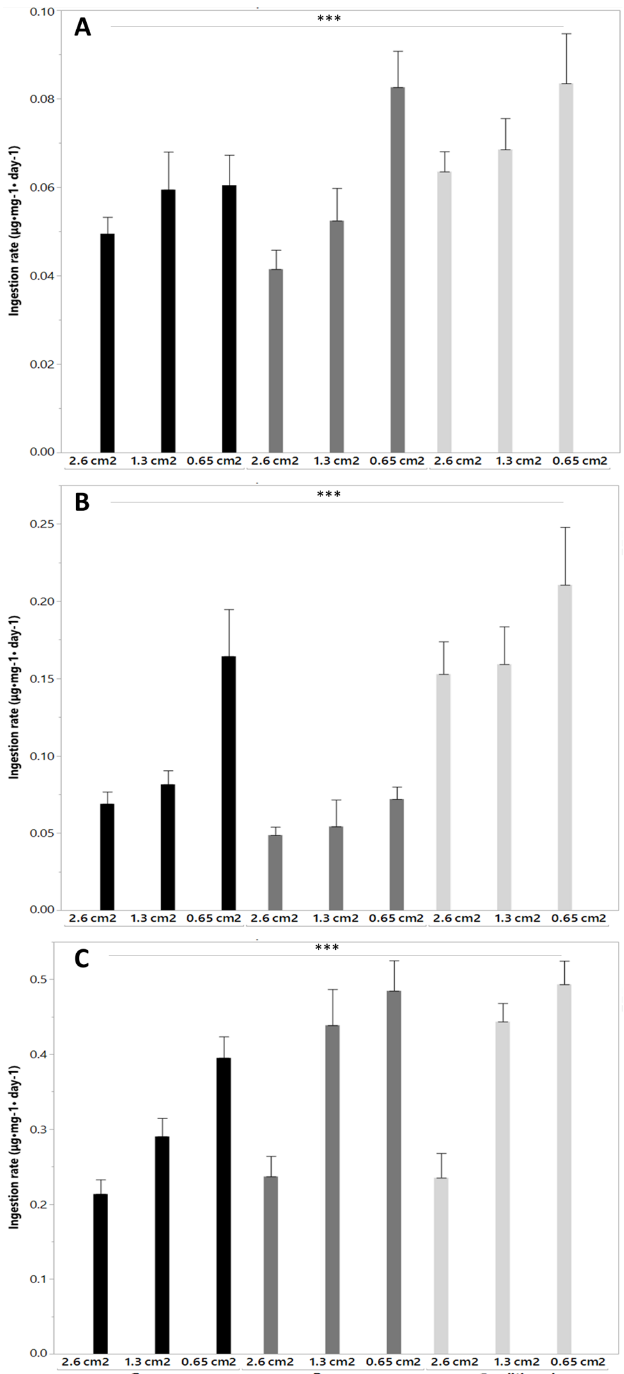

Fig. 4.4 – Mean (±SE) ingestion rate (µg•mg^{-1}• day^{-1}). A) *Piper glabrescens*, B) *Cecropia schreberiana*, C) *Spathodea campanulata* for each size area (0.65 cm^2, 1.3 cm^2 and 2.6 cm^2) and treatment (Green leaves, Brown leaves and Conditionate leaves). White bars – Conditionate leaves, Grey bars – Brown leaves, and Black bars – Green leaves. Horizontal line over the bars represents the ANOVA test differences NS- not significant; * $p< 0.05$; ** $p<0.01$; *** $p< 0.001$.

Table 4.1. Results of Three-way analyses of variance (ANOVAs) of plant species (*S. campanulata, C. schreberiana and P. glabrescens)* of three size area (0.65 cm^2, 1.3 cm^2 and 2.6 cm^2) and three conditionate treatment.

Source	df	Sum of squares	*F*	*p*
Size	2	1.99	49.84	<0.001***
Species	2	18.05	450.39	<0.001***
Treatment	2	0.65	16.14	<0.001***
Size x Species	4	1.62	20.24	<0.001***
Size x Treatment	4	0.07	0.83	0.506
Species x Treatment	4	0.82	10.17	<0.001***
Size x Species x Treatment	8	0.22	1.38	0.197

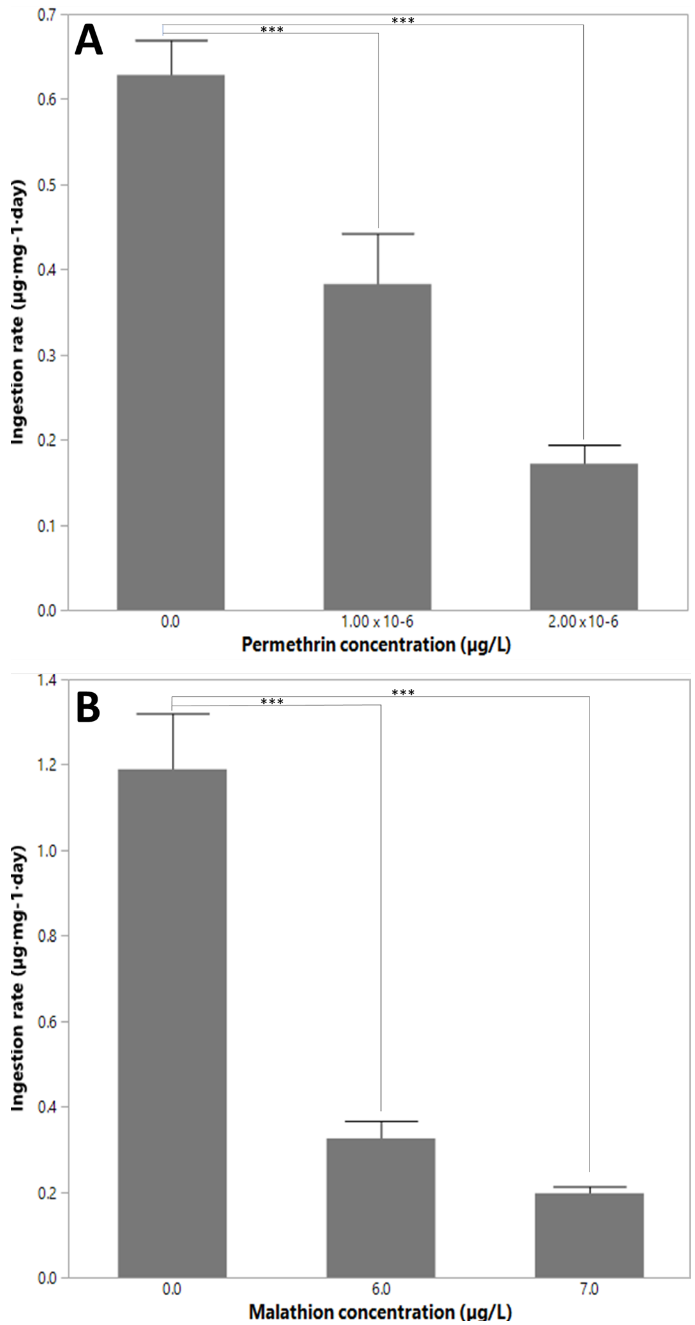

Fig 4.5 – Mean (±SE) ingestion rate (µg•mg^{-1}• day^{-1}). A) Permethrin exposure for two sublethal concentration (1.00 x 10^{-6} µg/L and 2.00 x 10^{-6} µg/L), B) Malathion exposure for two sublethal concentration (6.00 µg/L and 7.00 µg/L). Horizontal line over the bars represents the ANOVA test differences: NS- not significant; * $p< 0.05$; ** $p<0.01$; *** $p< 0.001$.

Table 4.2 – Mean (+ SE) of physicochemical for permethrin and malathion assay.

Treatment	Temperature (ºC)	Dissolved oxygen (mg. L^{-1})	pH	Turbidity (ppm)	Conductivity (μS.cm^{-1})
Control	20.6±0.03	8.9±0.1	7.4±0.01	145.9±0.1	282.2±0.1
Malathion	20.5±0.03	8.9±0.1	7.4±0.01	149.1±0.2	233.6±0.3
Permethrin	20.8±0.02	9.0±0.1	7.5±0.02	160.7±0.1	321.9±0.1

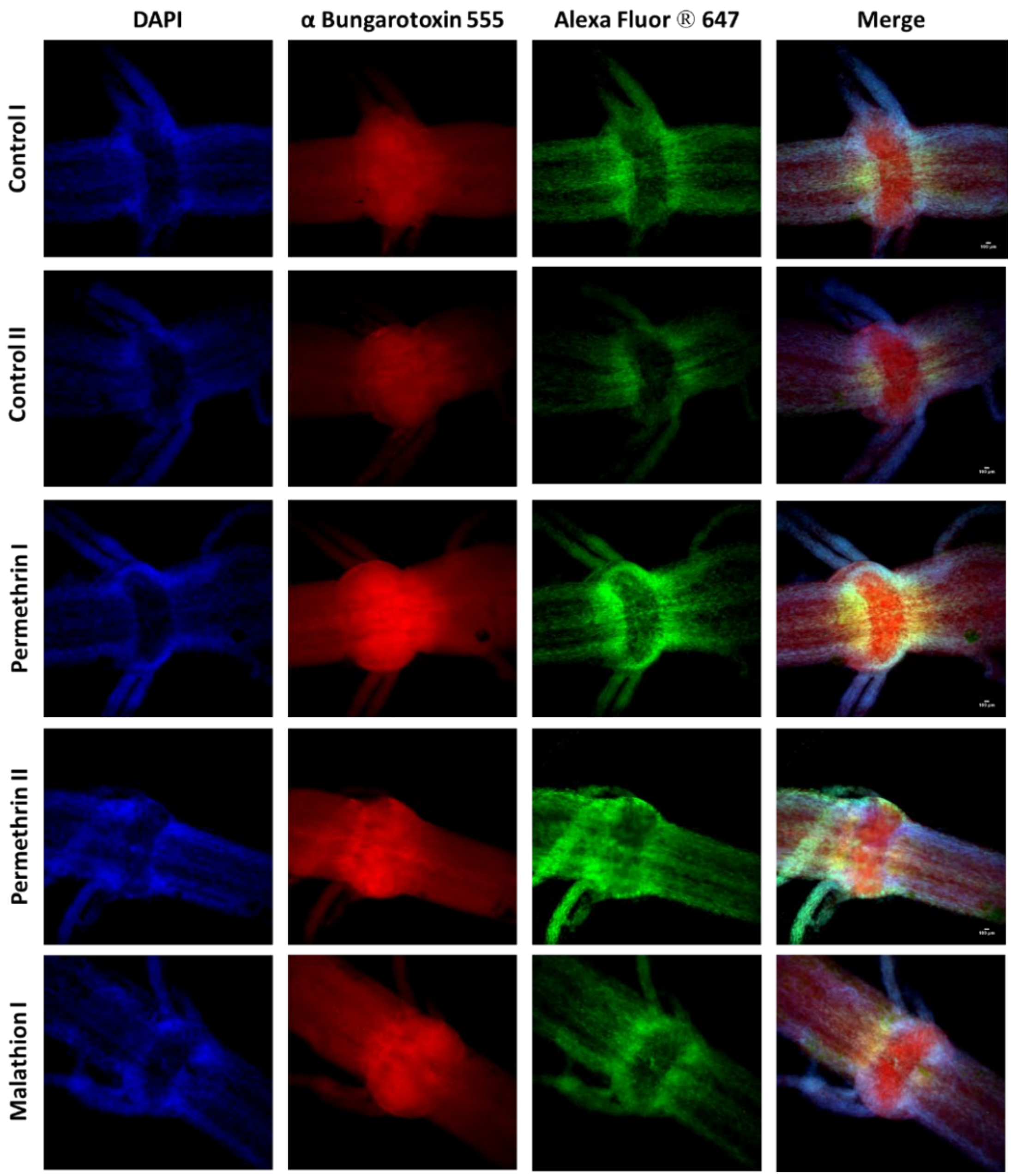

Fig 4.6 – Immunofluorescence of *X. elongata* ganglia after exposure of sublethal dose of permethrin and malathion. Epifluorescence Microscope; Objective: Olympus LUCPlanFLN 40x/0.60 ph2 ∞ /0-2/FN22; Camera: Andor Zyla; Immersion: Air; Scale bar: 100 μm. Each image shown was a representative of at least five cephalothorax ganglia. Blue channel – DAPI, Red channel- α-bungarotoxin 555; Green channel- Alexa Fluor ® 647.

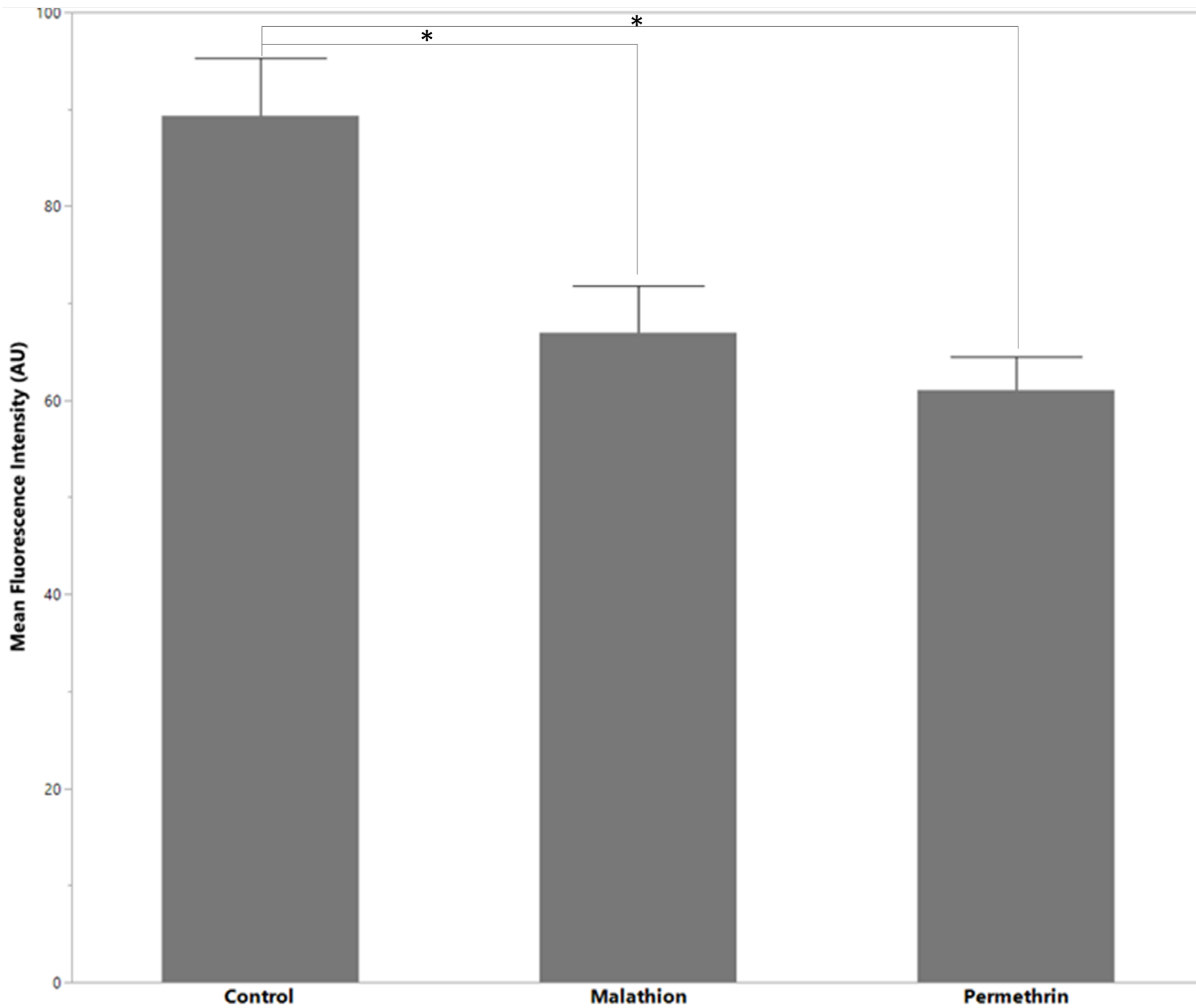

Fig 4.7 – Mean (±SE) fluorescent intensity (AU). Quantification of immunofluorescence staining for α-bungarotoxin revealed that mean fluorescent intensity for both pesticide exposed cephalothorax ganglia were significant different from control group. Permethrin (ANOVA, $F_{1,9} = 7.58$; $p<0.05$) and malathion (ANOVA, $F_{1,9} = 8.45$; $p<0.05$). Horizontal line over the bars represents the ANOVA test differences: NS- not significant; * $p< 0.05$; ** $p<0.01$; *** $p< 0.001$.

CHAPTER V

GENERAL CONCLUSION

The main objective of this thesis was to evaluate the toxicity of tropical freshwater shrimp to commonly used synthetic pyrethroid and organophosphate pesticides. To achieve this goal, several experiments were conducted in order to determine the effects of permethrin, malathion and glyphosate on different endpoints: survival, ingestion rates, and acetylcholinesterase activity (AChE). The model organism used in this study was the freshwater shrimp *Xiphocaris elongata* (Guérin-Ménevill, 1856) which plays a fundamental role in organic matter decomposition. In addition, this freshwater shrimp is a very important specie as they connect all functional groups of food webs (Covich & Mcdowell, 1996).

Urban activities are known to decrease the abundance of organisms living in freshwater environments (De Jesús-Crespo & Ramírez, 2011; Urban et al. 2006). In tropical streams, there are a variety of aquatic organisms including freshwater shrimp, fish, and macroinvertebrates that can be impacted by urban activities. Particularly, freshwater shrimp are vulnerable to the physical, chemical and ecological stressors changes of streams (Pérez-Reyes et al. 2016). However, it is not clear which environmental and anthropogenic variables are most important to explain the decrease in abundance of freshwater shrimp.

This thesis found that urban areas negative impact the abundance of the freshwater shrimp *Xiphocaris elongata* (Chapter II). In Río Sabana watershed, *X. elongata* abundance were best explained by temperature and pool canopy. This result agrees with previous studies that showed that higher water temperatures are associated with the reduction in canopy cover (Dugdale et al. 2018; Brasher, 2003). However, in Río Piedras watershed abundance of *X. elongata* were best explained by turbidity and pool depth. In urban areas, higher turbidities were found in deeper pools. These results are consistent with symptoms associated with the urban stream syndrome (Walsh et al. 2005). Previous study in Puerto Rico also found that increases in turbidity levels can have a negative effect on the migratory behavior of *Xiphocaris elongata* (Kikkert et al. 2009).

As part of urban activities, the uncontrolled application of organophosphate and pyrethroids pesticides like glyphosate, malathion, and permethrin has become a growing concern due to their ability to exert an adverse effect on a variety of non-targeted organisms. The majority of ecotoxicological studies have focus on a few selected model species, ignoring the effects on other non-target organisms which may play an important ecological role in tropical regions (Dam & Van den Brink, 2010; Gunnarsson & Castillo, 2018). The biological characteristics of aquatic crustaceans, including their morphology, physiology and behavior, make them susceptible to contamination by toxic chemicals (Rinderhagen et al. 2000).

This thesis found that acute toxicity for permethrin, malathion and glyphosate progressively increases as the concentration of exposure pesticide increased (Chapter III). We also found that the synthetic pyrethroid permethrin is the most toxic pesticide tested followed by the organophosphate malathion and glyphosate. Similar results were found in previous study that show that shrimp are highly susceptible to synthetic pyrethroid pesticides (Hook et al. 2018; Smith & Stratton, 1986). Even at sublethal concentrations, there could be significant behavioral changes, such as feeding behavior and locomotion activity, which may affect shrimp survival. This research also revealed that *X. elongata* is a suitable test organism, which can act as a representative of tropical freshwater environments.

In natural environments, decaying leaf material may accumulate contaminants, including insecticides and herbicides present in surface water (Englert et al. 2017; Kreutzweiser et al. 2007; Rasmussen et al. 2012). Thus, shredder shrimp can be exposed to these types of pesticide by leaf litter material (Forrow & Maltby, 2000; Wilding & Maltby, 2006). Exposure to contaminated leaf litter can decrease feeding rate reducing growth, size, fecundity, and survival of individuals (Anderson & Cummins,1979; Harmon & Wiley, 2010; Swan & Palmer, 2006). Organophosphate and pyrethroid pesticides, like malathion and permethrin, inhibits acetylcholinesterase (AChE) activity. Acetylcholinesterase is a critical enzyme in the normal function of the nervous system (Lionetto et al. 2011). The inhibition of this enzyme can result in the accumulation of the neurotransmitter acetylcholine in the synaptic gap leading to disruption of the nervous system.

In Chapter IV, we found that *Xiphocaris elongata* prefer certain size area and plant species when no-alternative were offered. The size area and plant species more appropriate for future toxicological studies is *Spathodea campanulata* leaves with a size area of 0.65 cm^2. This thesis also showed that sublethal concentrations of malathion and permethrin in leaves have a significant effect on ingestion rates. In addition, immunofluorescence in cephalothorax ganglia shown a decline in acetylcholinesterase activity when sublethal dose of malathion and permethrin increases. The observed results suggest that AChE activity can be used as biomarker for the detection and assessment of synthetic pyrethroids and organophosphate exposure on non-target species like the shredder shrimp *Xiphocaris* *elongata*. This study highlights the importance of shredders freshwater shrimp as potential bioindicator organisms to assess the risk of pesticide contamination in freshwater environments.

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