

Effect of Particle Size and Pesticide Contamination on Preference and Ingestion Rates by the Tropical Freshwater Shrimp *Xiphocaris elongata*

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How to cite this paper: Torres-Pérez, W.X. and Pérez-Reyes, O. (2023) Effect of Particle Size and Pesticide Contamination on Preference and Ingestion Rates by the Tropical Freshwater Shrimp *Xiphocaris elongata*. *Open Journal of Ecology*, 13, 183-198.

<https://doi.org/10.4236/oje.2023.134012>

Received: March 3, 2023

Accepted: April 7, 2023

Published: April 10, 2023

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Abstract

In tropical streams, freshwater shrimp are essential to preserve the structure and function of lotic ecosystems. Shredder shrimp play a fundamental role in organic matter decomposition because these feed on detritus. In addition, they are especially important organisms as they connect all trophic levels at food webs. In natural environments, decaying leaf material may accumulate contaminants, including insecticides and herbicides. At these, shredder shrimp can be exposed to these contaminants through ingestion of leaf litter material. The objectives of this study were to evaluate if the shredder shrimp *Xiphocaris elongata* display a preference for feeding on different plant species and leaf size areas 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 cm². This study also showed that sublethal concentrations of malathion and permethrin in leaves seem to have a significant effect on the ingestion rates of *X. elongata*, which suggests that the presence of these contaminants influenced feeding behavior. Immunofluorescence in cephalothorax ganglia showed a decline in AChE activity when the sublethal dose of malathion and permethrin increased. The observed results suggest that AChE activity can be used as a biomarker to detect and assess permethrin and malathion exposure on shredder shrimp.

Keywords

Malathion, Permethrin, Pesticide, Puerto Rico, *Spathodea*, Toxicology

1. Introduction

Freshwater shrimp are essential to preserve the structure and function of tropical lotic ecosystems. These organisms have an important role as biological indicators of stream health, and much is known about their ecology in the tropical island of Puerto Rico [1] [2] [3]. A total of 17 species from three families have been described for the island [4] [5]. 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 as wading birds [6] and diadromous predatory fishes [3] [7]. Thus, shredder organisms are especially important as they connect trophic levels at food webs. However, few toxicological studies have focused on tropical shredder species.

In this study, the shredder shrimp *Xiphocaris elongata* [8] was selected as the model organism. Shredder shrimp are essential processors of allochthonous organic carbon inputs and a food source for other aquatic and terrestrial organisms [9] [2] [10]. In natural environments, decaying leaf material may accumulate pollutants, including insecticides and herbicides present in surface water [11] [12] [13]. As a result, these shredder shrimps can be exposed to pesticides by ingesting contaminated leaf litter [14] [15]. 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 [16] [17] [18] [19], which can result in changes in the stream community's composition, structure, and function [14].

The ability of shredders to feed on leaf litter selectively suggests that foraging decisions these organisms make can be affected by contaminated leaves. In addition, exposure to contaminated leaf litter can decrease feeding rates, thus reducing individuals' growth, size, fecundity, and survival [20] [21] [22]. The ability of freshwater shredder organisms to use different species of leaf litter as a food source has been evaluated in many studies [23] [24] [25]. Most feeding preference studies focused on the importance of microbial colonization of leaf litter for palatability [26] [27]. However, shredder organisms can also use fresh and brown unconditioned leaves and are sometimes even preferred [28] [29] [30]. Leaves are one of the entry routes of pesticides inside benthic aquatic organisms because decaying leaf material can accumulate pollutants [31]. Organophosphate and pyrethroid pesticides, like malathion and permethrin, inhibit 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 [32].

The objectives of this study were to evaluate if the shredder shrimp *Xiphocaris elongata* prefers to feed on different leaf species and leaf size areas while also as-

sessing their consumption of leaves contaminated with pesticides. No-choice assays were performed to determine 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.

2. Materials and Methods

2.1. Collection and Acclimation of Organisms

Adults of *Xiphocaris elongata* (cephalothorax larger than 13.0 mm) were collected at Río Sabana near Sabana Field Research Station (18°19'29"N, 65°43'47"W) in Luquillo (elevation 113 above sea level), Puerto Rico. Freshwater shrimp were collected using an electrofishing backpack (Model 12-B, Smith-Root, Vancouver, Washington, USA) [33]. 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 [7] [3]. 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).

2.2. Leaves Collection and Preparation

Three common riparian species found in forested and urban streams on the island, the “trumpet-tree” *Cecropia schreberiana* [34], the “African tulip tree” *Spathodea campanulata* [35], and the “Guyanese pepper” *Piper glabrescens* [36] were used in this study. Green leaves were picked from trees and oven-dried at 55°C 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°C for 48 h. To mimic the conditioning history of leaf litter entering the streams, green leaves were picked from trees, air-dried overnight (55°C), 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 (~24°C - 30°C) for 12 h. All leaves were cut into squares of three different size categories (0.65 cm², 1.3 cm², and 2.6 cm²), weighed, and placed in aquariums for shrimp feeding trials.

2.3. 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 each shrimp only had one available size of pre-weighed leaf squares. Organisms (N = 50) were not allowed to feed 48 h prior to the beginning of the test. The exposure tanks (N = 25) (cylinder: di-

iameter—15.4 cm, height—15.4 cm) are assembled with two boxes: the upper box (length—10.6 cm, width—10.6 cm, height—10.6 cm), where the organism will be exposed, which has holes (0.5 cm diameter) in the bottom surface to prevent the contact of the organism with detritus and feces that fall into the bottom of the second box (**Figure 1(a)**). Control tanks (N = 25) (diameter—15.4 cm, depth 15.4 cm) only had leaf squares to evaluate autogenic changes in the absence of the organism. Each tank was filled with 2.0 L of dechlorinated water. The preference test was monitored daily for 96 h. During the test period, temperature ($^{\circ}\text{C}$), dissolved oxygen ($\text{mg}\cdot\text{L}^{-1}$), turbidity (ppm), conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$), and pH were recorded every day. At the end of the test, the remaining leaf squares were frozen for 24 h to eliminate further breakdown, oven-dried at 55°C for 48 h, and weighed to determine the feeding rate. All shrimp were measured and weighed before and after the exposure.

2.4. 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 experiments were not allowed to feed 48 h prior to the beginning of the test. In the experimental tanks, one adult shrimp with the pre-weighed leaf squares contaminated with pesticides were added (**Figure 1(b)**). Shrimp were placed in the tanks five days previous to the treatment to facilitate animal acclimation. For each pesticide, two sublethal (nominal) concentrations of permethrin ($1.00 \times 10^{-6} \mu\text{g}\cdot\text{L}^{-1}$ and $2.00 \times 10^{-6} \mu\text{g}\cdot\text{L}^{-1}$) and malathion ($6.00 \mu\text{g}\cdot\text{L}^{-1}$ and $7.00 \mu\text{g}\cdot\text{L}^{-1}$) were used. At the end of the experiments, leaf squares were frozen for 24 h to eliminate further breakdown and oven-dried at 55°C for 48 h. Finally, they were

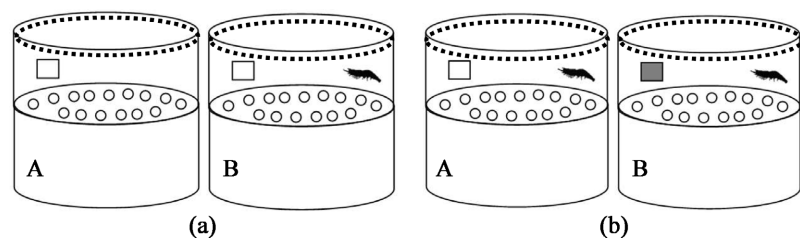


Figure 1. (a) Exposure tanks assemble. (A) Control tanks only had leaves square to evaluate autogenic changes in the absence of the organism. (B) Experimental tanks had a leaf square with the shrimp to evaluate the ingestion rate. The upper chamber has holes to prevent contact of the organism with detritus that fall into the bottom of the second chamber. White square—leaf. Size of the shrimp ≤ 13 mm cephalothorax length. Dotted line represents the water level—2 L; the upper box dimensions: length—10.6 cm, width—10.6 cm, height—10.6 cm, where the organism will be exposed, which has holes of 0.5 cm diameter. (b) Exposure tanks with two chambers were used to evaluate changes in ingestion rate between uncontaminated and contaminated leaves. Organisms for these tests were not allowed to feed 48 h 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.

weighed to determine the feeding rate. All shrimp used were measured and weighed before and after exposure. During the test period, temperature, dissolved oxygen, turbidity, conductivity, and pH were measured daily. Ingestion rates were calculated using the following formula: $I = ((\Delta Lw)/(Lwi))(1/TShw)$; where, ΔLw represents the changes of leaves weight in μg ($Lwi - Lwf$); Lwi is the initial leaf weight; T is the time expressed in days, and Shw (mg) is the shrimp weight. Ingestion rate is reported in $\mu\text{g}\cdot\text{mg}^{-1}\cdot\text{day}^{-1}$.

2.5. Effects of Pesticides in Nervous Cord: Immunofluorescence and Image Analysis

In total, 30 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% (v/v) paraformaldehyde. The nervous cords were divided into segments to separate the cephalothorax ganglia from the abdominal ganglia. Cephalothorax ganglia were permeabilized in HBSS/Sucrose + 0.1% (v/v) saponin solution (HBSS:Su:Sap) for 10 min and blocked in 5% fetal bovine serum (FBS) diluted in HBSS:Su:Sap for 15 min, followed by α -Bungarotoxin, Alexa Fluor™ 555 conjugate diluted in HBSS:Su:Sap: FBS serum for one 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 one hour at RT, followed by the nuclear counterstain DAPI (ThermoFisher Scientific/Molecular Probes, Cat. #D1306, Lot. #2031179) (10 μM) diluted in HBSS for one hour at RT. Images were taken on an Olympus Inverted Epifluorescence Microscope with an objective Olympus LUCPlanFLN 40-/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.

2.6. Statistical Analyses

The statistical analyses were performed in JMP Pro version 17 Software Software SAS Institute Inc., Cary, NC, USA [37]. Factorial analyses of variance (ANOVA) were used to test the effects of plant species, leaf area, and exposure treatment. In addition, one-way analysis of variance (ANOVA) was used to compare total ingestion rates of contaminated vs uncontaminated leaves and mean fluorescence intensities from the immunofluorescent experiment. A post hoc Tukey's test was used to identify treatment groups with highly significant differences ($P < 0.05$).

3. Results

3.1. Leaf Area and Plant Species Preference

Physicochemical parameters measured during the leaf area and plant species preference assays were: temperature $20.9^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$, conductivity $349 \pm 1.80 \mu\text{S}$, pH 7.81 ± 0.01 , dissolved Oxygen $9.01 \pm 0.01 \text{ mg}\cdot\text{L}^{-1}$, and turbidity $174 \pm 0.69 \text{ ppm}$. Significant differences (Factorial ANOVA, $F_{(2,39)} = 49.84$; $P < 0.001$) were found for the ingestion rates of the different leaf sizes (**Table 1**; **Figure 2**). Leaves with an area of 0.65 cm^2 were ingested in higher quantity for all plant species. Significant differences (Factorial ANOVA, $F_{(2,39)} = 450.39$; $P < 0.001$) were also found for the different plant species in the preference test. Conditionated leaves of *Spathodea campanulata* were preferred and ingested in a higher quantity ($0.49 \pm 0.03 \mu\text{g}\cdot\text{mg}^{-1}\cdot\text{day}^{-1}$). Brown *Piper glabrescens* leaves were the least ingested ($0.041 \pm 0.004 \mu\text{g}\cdot\text{mg}^{-1}\cdot\text{day}^{-1}$) (**Figure 3**).

3.2. 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 (**Figure 4**). Higher concentration of permethrin had a lower ingestion rate than the control group ($0.64 \pm 0.05 \mu\text{g}\cdot\text{mg}^{-1}\cdot\text{day}^{-1}$). In addition, the ingestion rates of malathion-contaminated leaves were also statically different from the control group (ANOVA, $F_{(2,24)} = 24.281$; $P < 0.001$; **Figure 4**). Physicochemical parameters for the permethrin and malathion assays are summarized in **Table 2**.

3.3. Effects of Pesticides in Nervous Cord: Immunofluorescence and Image Analysis

Control and pesticide exposed shrimp ganglia were stained with α -bungarotoxin to label acetylcholinesterase (AChE) at the cephalothorax segments (**Figure 5**). α -Bungarotoxin staining appeared qualitatively similar in both control and pesticide exposed ganglia. However, the mean fluorescence intensity was statistically

Table 1. Results of Factorial analyses of variance (ANOVA) of plant species (*S. campanulata*, *C. schreberiana* and *P. glabrescens*) of three size areas (0.65 cm^2 , 1.3 cm^2 and 2.6 cm^2) and three conditionate treatments. df-degree of freedom; NS-not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Source	df	Sum of Square	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 \times Species	3	1.62	20.24	<0.001***
Size \times Treatment	3	0.07	0.83	0.506 NS
Species \times Treatment	3	0.82	10.17	<0.001***
Size \times Species \times Treatment	8	0.22	1.38	0.197 NS

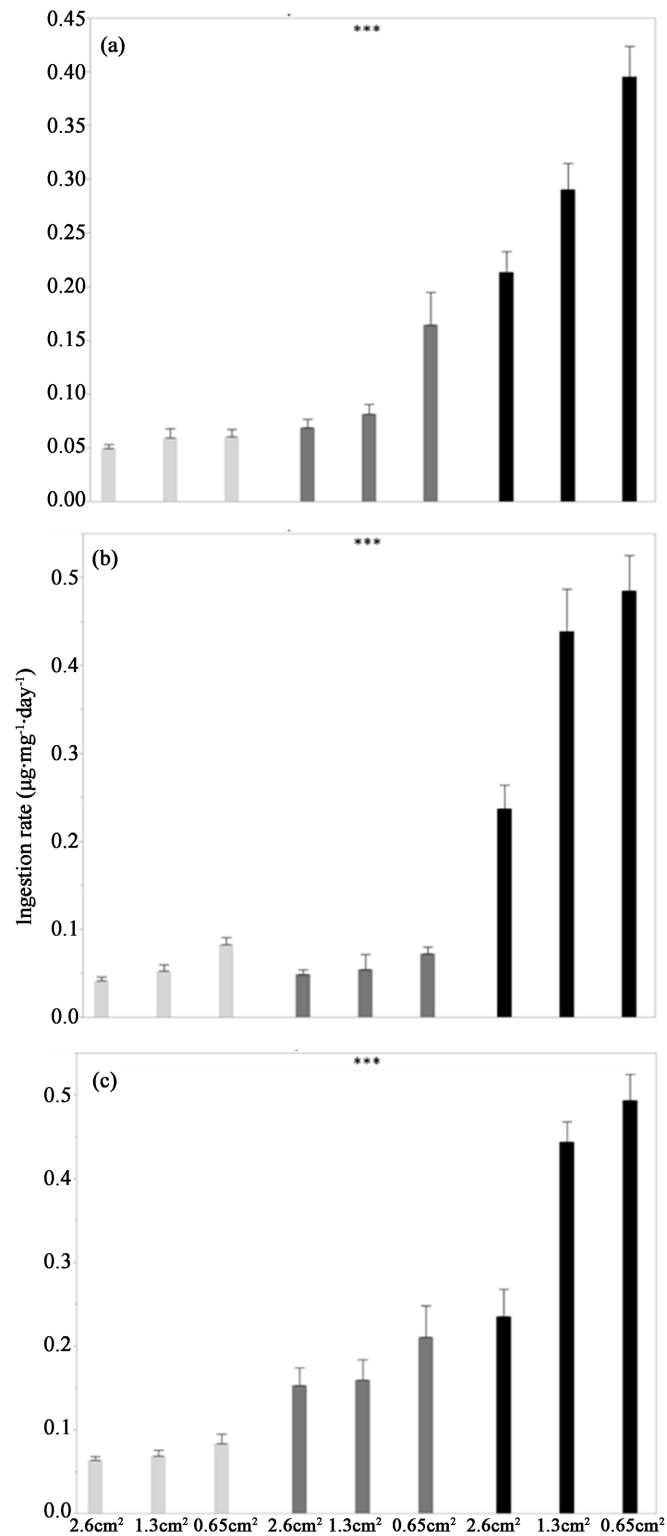


Figure 2. Mean (\pm SE) ingestion rate ($\mu\text{g}\cdot\text{mg}^{-1}\cdot\text{day}^{-1}$). (a) Green leaves, (b) Brown leaves, (c) Conditioned leaves for each size area (0.65 cm^2 , 1.3 cm^2 and 2.6 cm^2) and plant species (*S. campanulata*, *C. schreberiana* and *P. glabrescens*). White bars—*P. glabrescens*, Grey bars—*C. schreberiana*, and Black bars—*S. campanulata*. Horizontal line over the bars represents the ANOVA test differences NS- not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

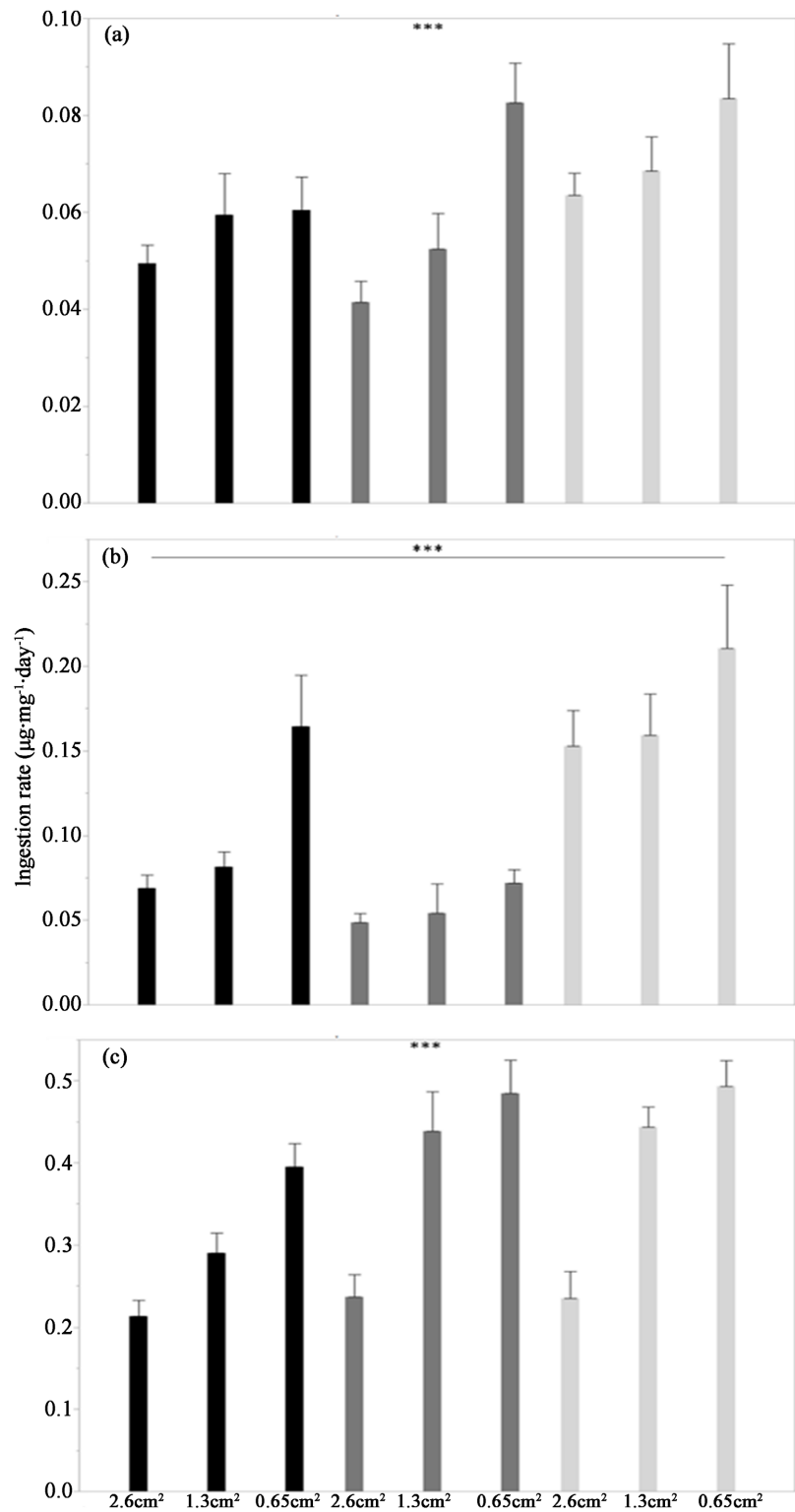


Figure 3. Mean (\pm SE) ingestion rate ($\mu\text{g}\cdot\text{mg}^{-1}\cdot\text{day}^{-1}$). (a) *Piper glabrescens*, (b) *Cecropia schreberiana*, (c) *Spathodea campanulata* for each size area (0.65 cm², 1.3 cm² and 2.6 cm²) and treatment (Green leaves, Brown leaves, and Conditionate leaves). White 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.

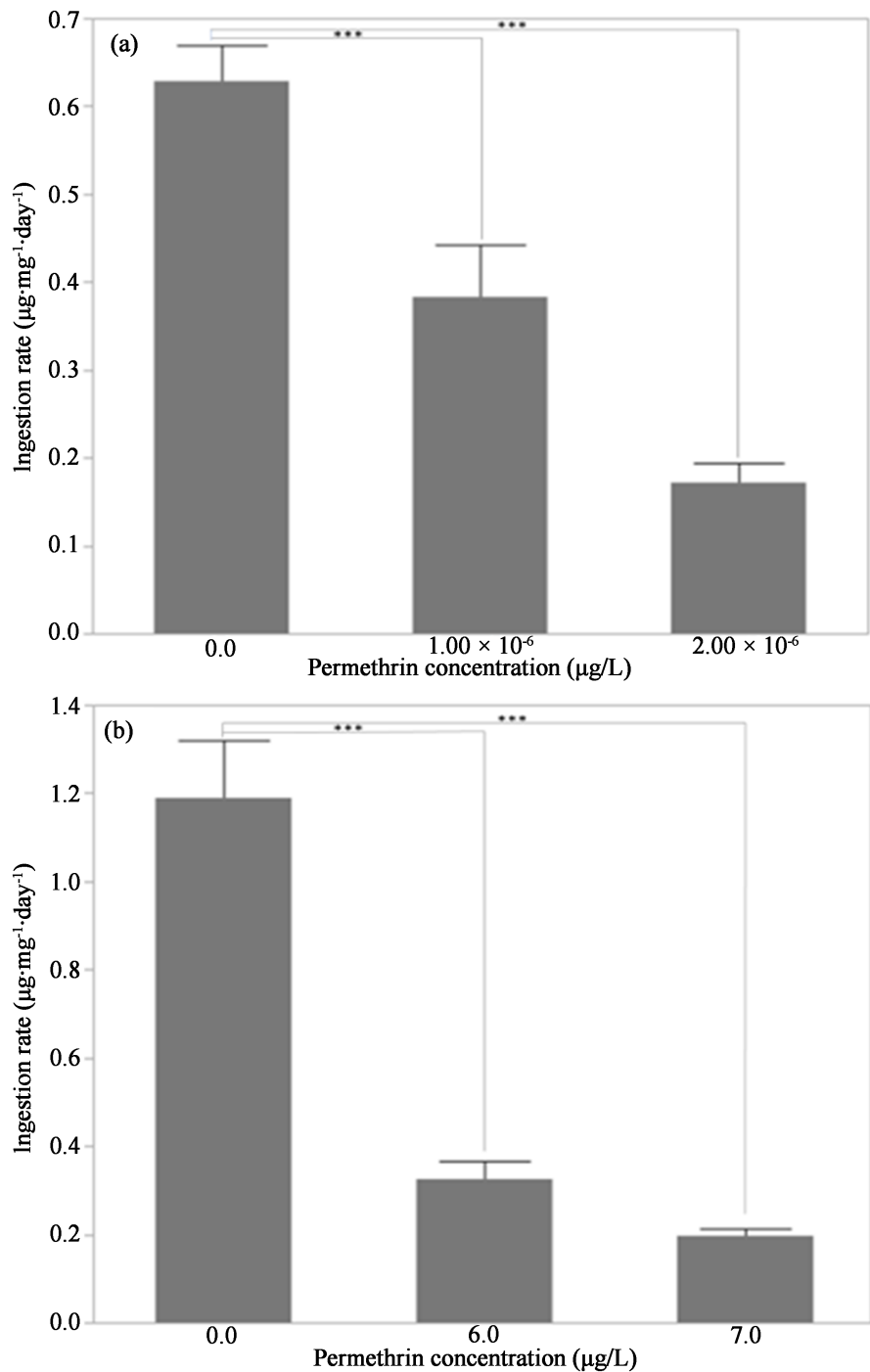


Figure 4. Mean (\pm SE) ingestion rate ($\mu\text{g}\cdot\text{mg}^{-1}\cdot\text{day}^{-1}$). A) Permethrin exposure for two sublethal concentrations (1.00×10^{-6} $\mu\text{g/L}$ and 2.00×10^{-6} $\mu\text{g/L}$), B) Malathion exposure for two sublethal concentrations (6.00 $\mu\text{g/L}$ and 7.00 $\mu\text{g/L}$). Horizontal line over the bars represents the ANOVA test differences: NS-not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

different from the control, permethrin (One-way ANOVA, $F_{(1,9)} = 7.58$; $P < 0.05$), and malathion (One-way ANOVA, $F_{(1,9)} = 8.45$; $P < 0.05$) exposed ganglia indicating that AChE activity could be affected by these pesticides.

Table 2. Mean (\pm SE) of physicochemical for permethrin and malathion assays.

Treatment	Physicochemical parameters				
	Temperature ($^{\circ}$ C)	Dissolved Oxygen ($\text{mg}\cdot\text{L}^{-1}$)	pH	Turbidity (ppm)	Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)
Control	20.6 \pm 0.03	8.9 \pm 0.1	7.4 \pm 0.01	145.9 \pm 0.1	282.2 \pm 0.1
Malathion	20.5 \pm 0.03	8.9 \pm 0.1	7.4 \pm 0.01	149.1 \pm 0.2	233.6 \pm 0.3
Permethrin	20.5 \pm 0.02	9.0 \pm 0.1	7.5 \pm 0.02	160.7 \pm 0.1	321.9 \pm 0.1

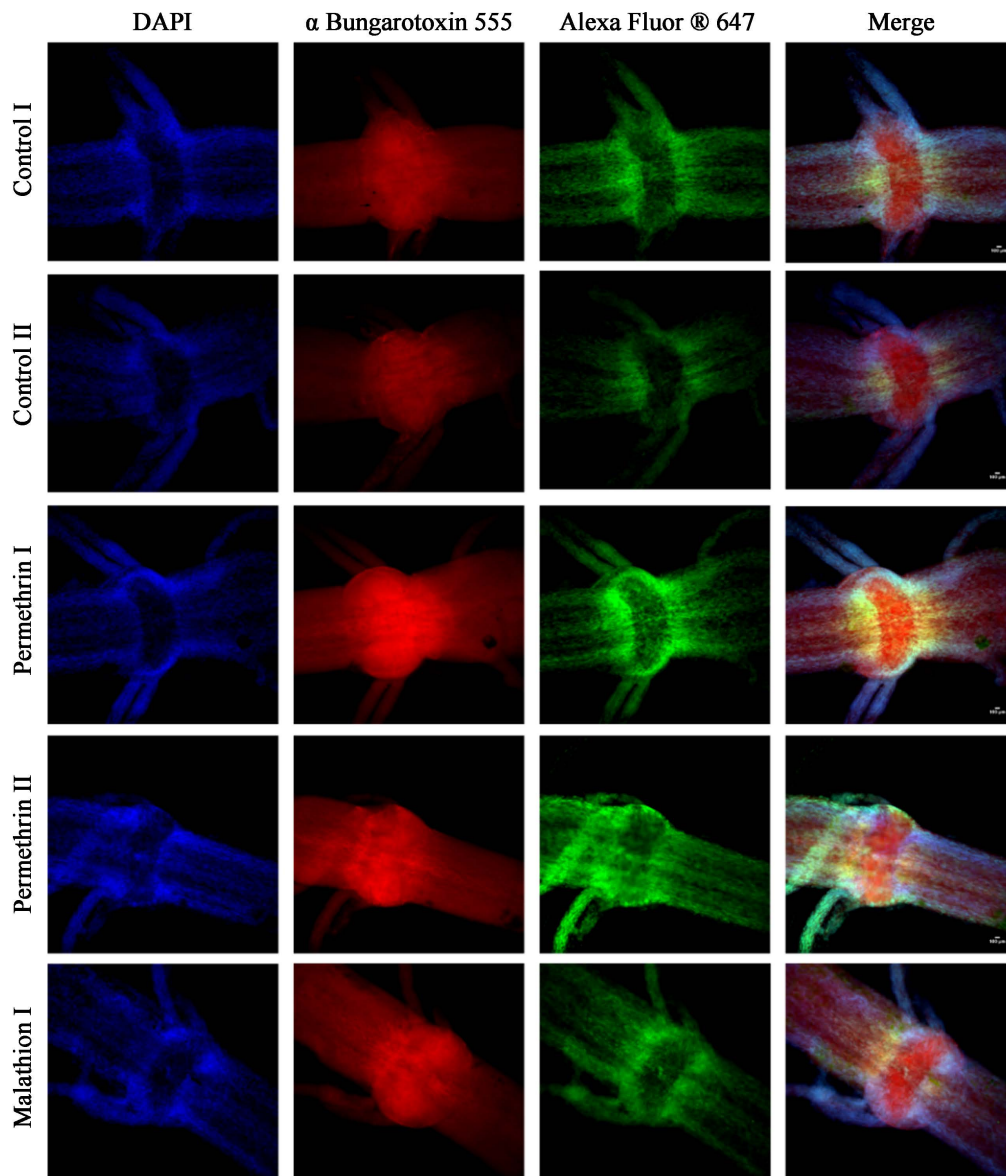


Figure 5. Immunofluorescence of *X. elongata* ganglia after exposure of sublethal dose of permethrin and malathion. Epifluorescence Microscope; Objective: Olympus LUCPlanFLN 40 \times /0.60 ph2 ∞ /0-2/FN22; Camera: Andor Zyla; Immersion: Air; Scale bar: 100 μm . Each image shown was a representative of at least five cephalothorax ganglia. Blue channel—DAPI, Red channel— α -bungarotoxin 555; Green channel—Alexa Fluor[®] 647. Quantification of immunofluorescence staining for α -bungarotoxin re-vealed 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, $F_{(1,9)} = 8.45$; $P < 0.05$).

4. 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 [38]. Thus, one of the objectives of this study was to determine if the shredder shrimp *Xiphocaris elongata* demonstrated a preference for feeding on riparian plant species and leaf areas. These results contribute to establish a leaf area and plant 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, 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 a smaller area than bigger leaf areas (2.6 cm²), resulting in a lower ingestion rate for all three plant species. This study demonstrated that *Spathodea campanulata* leaves, with an area of 0.65 cm², presented 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 [31]. As shredder organisms, *X. elongata* feeding on detritus is 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 [39] [40]. Given that some types of pesticides are present in surface waters, evaluating if these contaminants affect feeding behavior under different concentrations becomes urgent. Based on the results from assays where contaminated and uncontaminated leaves were offered to the shrimp, it was evident that the increasing concentration of permethrin and malathion on leaves significantly affected shrimp feeding behavior.

For permethrin, the ingestion rate on leaves for *X. elongata* decreases significantly at lower and higher concentrations of pesticides 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 ($1.00 \times 10^{-6} \mu\text{g}\cdot\text{L}^{-1}$) compared to the 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 [41]. Similarly, Armstrong and Bonner [42] found that permethrin exposure significantly reduced the feeding behavior of the fruit fly *Drosophila melanogaster*.

In the malathion assay, an increase in the 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 influenced feeding behavior. In the present study, the ingestion rate was significantly lower when leaves were spiked with a high ($7.0 \mu\text{g}\cdot\text{L}^{-1}$) and low ($6.0 \mu\text{g}\cdot\text{L}^{-1}$) the concentration of malathion. Similar results were found in a previous study using the shrimp *Macrobrachium nipponense* as a model organism. They found that malathion significantly reduced feeding rates as the pesticide concentration increased [43]. Considering the feeding assay results for the shrimp *X. elongata*, the ingestion rates seem to be affected by the presence of leaves having a high concentration of pesticides. The presence of pyrethroids and organophosphorus pesticides 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 [32]. 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 decreased in other species of shrimp exposed to sublethal concentrations of malathion [44]. Another study found similar results that demonstrated that malathion caused a reduction in *Daphnia magna* AChE activity levels by up to 50% with an adverse effect on mobility [45]. For permethrin, few studies have been reported regarding the effect on AChE activity. However, Khazri among others [17], showed that AChE activity in the 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 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 pesticides. For example, Jebali among others [46], after administering sublethal concentrations of malathion, observed a decrease in AChE activity, after two and seven days of the treatment, compared to controls in the brain tissue of the fish *Seriola dumerilli*. Chandra [47] showed maximum inhibition of 77.12% and 72.83% in the brain and gills, respectively, after the exposition of the freshwater catfish *Heteropneustes fossilis* to malathion. Moreover, Ibrahim among others [48] found that AChE activity in the dipteran *Chironomus riparius* significantly declines when the animals are exposed to permethrin.

5. Conclusion

Overall, the results showed that the shrimp *Xiphocaris elongata* preferred a particular leaf area and plant species when no alternatives were offered. The leaf area and plant species more appropriate for future toxicological studies are *Spathodea campanulata* leaf with an area of 0.65 cm^2 . This study also showed that

sublethal concentrations of malathion and permethrin in leaves seem to have a significant effect on the ingestion rates of *X. elongata*, which suggests that the presence of these contaminants influenced 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 a biomarker to detect and assess 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 tropical freshwater environments.

Acknowledgements

We are incredibly grateful to our many colleagues, especially Alan P. Covich and Todd A. Crowl, for their collaboration on studies of freshwater shrimp in Puerto Rico.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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