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Sublethal exposure to pyriproxyfen does not impair the abilities of the backswimmer *Buenoa amnigenus* to prey upon *Aedes aegypti* larvae

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Abstract

Pyriproxyfen is a juvenile hormone analogue that is commonly used to control the immature stages of mosquitoes in both artificial and natural water reservoirs. Recently, concerns have been raised regarding the community effectiveness of pyriproxyfen in preventing vector-transmitted diseases. Such concerns have been based on the unintended effects on non-target organisms and the selection of resistant mosquito populations. This investigation was, therefore, conducted to evaluate the toxicity of pyriproxyfen to *Aedes aegypti* (Diptera: Culicidae) larvae and the backswimmer *Buenoa amnigenus* (Hemiptera: Notonectidae), a naturally occurring mosquito larvae predator. We also assessed the abilities of backswimmers exposed to sublethal levels of pyriproxyfen to prey upon mosquito larvae (L2) under three larval densities (3, 6, or 9 larvae/100 mL of water) using artificial containers. Our results revealed that pyriproxyfen killed backswimmers only at concentrations higher than 100 µg active ingredient [a.i.]/L, which is 10 times higher than that recommended for larvicidal field application (i.e, 10 µg a.i./L). The abilities of backswimmers exposed to sublethal levels of pyriproxyfen (100 µg a.i./L) to prey upon mosquito larvae were not affected. Harmful effects on the backswimmer predatory abilities were detected only at concentrations of 150 µg a.i./L and when there was a higher prey availability (i.e., 9 larvae/100 mL of water). Together, our findings indicate that the reduced community effectiveness of this insecticide derives from factors other than its detrimental effects on non-target organisms such as backswimmers.

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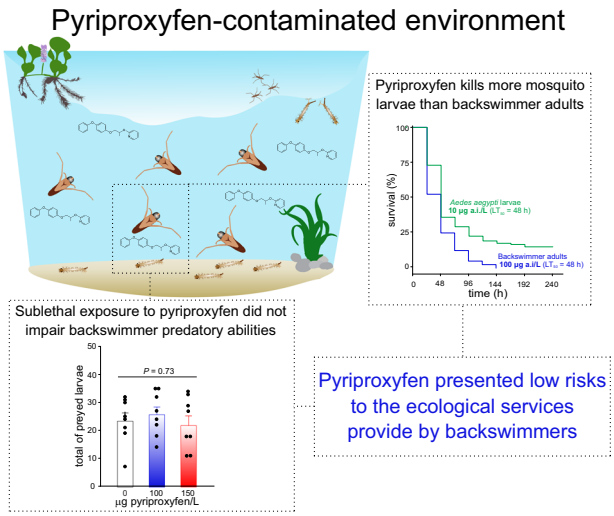

Keywords Non-target organism · Insecticide susceptibility · Insect growth regulator · Yellow fever mosquito

Introduction

The use of larvicidal insecticides to control mosquitoes that transmit human diseases such as Zika, dengue fever, and chikungunya has proven to be very successful (Darriet et al. 2010; Ohba et al. 2013; Maoz et al. 2017; Marina et al. 2018; Roiz et al. 2018; WHO 2020). Pyriproxyfen is the most commonly used larvicide in both artificial and natural water reservoirs globally (Darriet et al. 2010; Ohba et al. 2013; WHO 2013). It is a mimic of the natural juvenile hormone which can disrupt embryonic and larval insect development and impede adult emergence, leading to an effective reduction of mosquito populations (Sullivan and Goh 2008). Previous studies, however, have reported the harmful effects of pyriproxyfen on non-target organisms at concentrations found in the environment (Devillers 2020; Moura and Souza-Santos 2020).

The exposure to pyriproxyfen of non-target organisms (e.g., larvivorous fish and microcrustaceans) that inhabit the same breeding sites as mosquitoes can lead to reduced locomotion in a number of these species and have raised concerns regarding the toxicity of pyriproxyfen (Caixeta et al. 2016; Truong et al. 2016; Vieira Santos et al. 2017). An increasing number of reports demonstrating similar unintended effects of pyriproxyfen brings into question its effects on community effectiveness, selection of resistant mosquito populations, and detrimental effects on non-target aquatic invertebrates (Maoz et al. 2017; Vieira Santos et al. 2017; Devillers 2020; Valbon et al. 2021). Hence, such unintended effects may impact the ecological services provided by naturally occurring aquatic predators of mosquito larvae (Antwi and Reddy 2015; Valbon et al. 2018).

Among the aquatic insects endangered by unintended pyriproxyfen exposure, the semiaquatic backswimmer (Hemiptera: Notonectidae) represents an interesting model organism, as these insects function not only as mosquito predators, but also as prey of vertebrates and other invertebrates, contributing to shape community structure (Van de Meutter et al. 2008; Shaalan and Canyon 2009; Secondi and Raux 2020). Backswimmers are cosmopolitan predators and are among the first insects to colonize freshwater habitats, such as artificial and natural water reservoirs (Giller and McNeill 1981), which increases their likelihood of encountering insecticide-contaminated ecosystems. Both nymph and adult backswimmers are primarily sit-and-wait surface predators (i.e., ambush strategy), that can also actively explore the water column using their forelegs to rapidly capture prey (Giller and McNeill 1981; Gutiérrez et al. 2017b).

Recent studies have suggested the use of aquatic insects as biological agents for controlling mosquito larvae (including *Aedes* spp.) in both confined and natural environments (Shaalan and Canyon 2009; Sivagnaname 2009; Kweka et al. 2011; Eba et al. 2021). For instance, backswimmers have been shown to be the more aggressive predators of *Anopheles* spp. (Diptera: Culicidae) mosquito larvae compared to dragonflies (Odonata: Libellulidae) and water bugs (Hemiptera: Belostomatidae) (Eba et al. 2021). Thus, biological control agents may be alternatives not only to be preserved/employed in integrated mosquito management strategies but also when combined with insecticides (Roiz et al. 2018). It remains to be determined, however, whether pyriproxyfen causes detrimental effects on the predatory behaviour of backswimmers.

Here, based on its harmful effects to non-target organism (Caixeta et al. 2016; Truong et al. 2016; Maoz et al. 2017; Vieira Santos et al. 2017; Devillers 2020; Moura and Souza-Santos 2020), we hypothesized that pyriproxyfen has toxic effects on backswimmers. Further, sublethal pyriproxyfen exposure can alter the predatory abilities of backswimmers to prey upon mosquito larvae. This insecticide/predator/prey interaction may represent a threat to backswimmers that are capable of coexisting in mosquito breeding sites (Arredondo-Jimenez and Valdez-Delgado 2006; Shaalan and Canyon 2009). We utilised both toxicological and behavioral approaches to investigate the impact of pyriproxyfen on the backswimmer *Buenoa amnigenus* (White) (Hemiptera: Notonectidae), a species widely distributed in Brazil (Ribeiro et al. 2022). We used yellow fever mosquito *Aedes aegypti* (L.) (Diptera: Culicidae) as the model organism due to its widespread presence in simple aquatic communities and the potential to be preyed upon by generalist predators (Shaalan and Canyon 2009; Sivagnaname 2009). Specifically, we evaluated (1) whether pyriproxyfen caused similar mortality in *A. aegypti* larvae (target organism) and *B. amnigenus* adults (non-target organism), (2) whether *B. amnigenus* adults recovered from the exposure to sublethal levels of pyriproxyfen, and (3) whether backswimmer adults exposed to sublethal levels of pyriproxyfen showed impaired performance to prey upon mosquito larvae at different prey densities.

Our findings suggest that pyriproxyfen is more toxic to *A. aegypti* larvae than *B. amnigenus* adults. Harmful effects on the predatory behavior of *B. amnigenus* were observed only at high concentration of pyriproxyfen (150 µg a.i./L) and when these predators faced high larval availability. Given its efficiency in controlling mosquito populations at concentrations as low as 10 µg a.i./L, our findings indicate that pyriproxyfen may be a safe option for implementation of mosquito management strategies (e.g., use of selective insecticides) that preserve mosquito larvae predators in freshwater ecosystem.

Materials and methods

Insects

We used second instar (L2) *A. aegypti* larvae of an insecticide-susceptible strain (PPCampos, originally collected at Campos dos Goytacazes, Rio de Janeiro State, Brazil). Larvae were maintained in dechlorinated tap water and fed on turtle food daily (Reptolife, Alcon Pet, Camburiú, SC, Brazil), and under controlled conditions (temperature: 25 ± 2 °C, relative humidity: 60 ± 2%, and photoperiod of 12 h of light) (Haddi et al. 2017; Mendes et al. 2017). Adult *B. amnigenus*, were collected from fish farming installations at the Federal University of Viçosa (UFV, Viçosa, MG, Brazil, 20°45′ S, 42°52′ W). Before experiments, the insects were acclimatized by maintaining groups of 50 backswimmers in 600 mL glass beakers containing 500 mL of dechlorinated tap water (conductivity of 425 ± 30.5 µS/cm and pH of 7.4 ± 0.1) for 24 h under controlled conditions (25 ± 2 °C, 12 h of photophase) (Gutiérrez et al. 2017a). A total of five beakers (totalizing 250 backswimmers) were used for acclimatization. Identification of male specimens of *B. amnigenus* anaesthetized with ice (for five minutes) was carried out under a stereomicroscope following an appropriate key (Truxal 1952). Although we used males for identification, both male and female (approximately 4.7–5.4 mm and 4.8–5.9 mm, respectively) were randomly selected and used in the toxicological and predation bioassays. It is worth noting that *B. amnigenus* occur sporadically in natural water reservoirs and always aggregated, which makes it possible to collect many individuals of the same species. We conducted at least three rounds of field collection at the same location at different intervals to assure our identification.

Toxicological bioassays

We examined the susceptibility of *A. aegypti* larvae and *B. amnigenus* adults to pyriproxyfen [100 g active ingredient (a.i.)/L, emulsifiable concentrate, Sumitomo Chemical Ltda, São Paulo, Brazil] at different nominal concentrations (ranging from 10 µg a.i./L to 500 µg a.i./L). For all toxicological bioassays we ran a single-pulse test using pyriproxyfen. Recent studies have shown that pyriproxyfen decreases over 50% within the first 24 h and maintains the same concentration for five consecutive days (Caixeta et al. 2016; Vieira Santos et al. 2017; Valbon et al. 2021). The control treatments were conducted exposing the insects (*A. aegypti* larvae or *B. amnigenus* adults) to dechlorinated tap water (conductivity of 425 ± 30.5 µS/cm and pH of 7.4 ± 0.1) for all toxicological bioassays. All toxicological bioassays were performed at controlled temperature (25 ± 2 °C), humidity (60 ± 2%) and photoperiod (12 h of light phase) conditions.

Aedes aegypti larvae

We conducted a survival bioassay where *A. aegypti* larvae were exposed to concentrations of pyriproxyfen recommended for their control in field applications (i.e., 10 and 20 µg a.i./L). Groups of 25 mosquito larvae (L2) were transferred to glass beakers containing 100 mL of insecticide. For each insecticide concentration treatment, we used 100 mosquito larvae (i.e., repetition). Mortality was assessed every 24 h for 10 consecutive days under pyriproxyfen solution and insects that remained motionless after being

repeatedly stimulated mechanically with a pipette were considered dead.

Buenoa amnigenus adults

We used three independent experimental sets in order to assess the toxicity of pyriproxyfen to backswimmer adults:

(i) Concentration-mortality bioassay: *B. amnigenus* were exposed to concentrations of pyriproxyfen that ranged from 50 to 500 µg a.i./L, which caused 5–95% mortality. In brief, four replicates (groups of 10 insects) were used for each concentration. Groups of 10 adult backswimmers were placed into glass beakers containing 300 mL of insecticide. All beakers were covered with organza fabric to prevent insect escape. Mortality was assessed only once (after 24 h of exposure) and insects that remained motionless after being repeatedly stimulated mechanically with a pipette were considered dead (Gutiérrez et al. 2017a).

(ii) Survival assay without recovery: *B. amnigenus* were exposed to pyriproxyfen at concentrations of 100 and 150 µg a.i./L, which corresponded to the sublethal concentrations LC_1 and LC_{10} estimated by concentration-mortality analysis. For that, groups of 10 adult backswimmers were placed into four glass beakers containing 300 mL of insecticide. For each insecticide concentration treatment, we used 40 backswimmers (i.e., repetition). Mortality was assessed every 24 h for 10 consecutive days under pyriproxyfen solution and insects that remained motionless after being repeatedly stimulated mechanically with a pipette were considered dead.

(iii) Survival assay with recovery: To assess the ability of *B. amnigenus* to recover from sublethal pyriproxyfen exposure, we conducted a survival bioassay (as above described) with individuals that were previously exposed for 24 h to pyriproxyfen at concentrations of 100 and 150 µg a.i./L. After 24 h of pyriproxyfen exposure, backswimmers were transferred to dechlorinated tap water and mortality was assessed every 24 h for 10 consecutive days. It is worth noting that there was no insect mortality in our unexposed (control) treatments (Figs. 1A and 2, control treatment in black lines), indicating that no naturally occurring deaths in the backswimmers or the larvae in the first 24 h of experiment.

Predation bioassays with backswimmer adults

To assess the abilities of backswimmers exposed to sublethal levels of pyriproxyfen to prey upon *A. aegypti* larvae (L2), adults were exposed to pyriproxyfen at concentrations of 100 µg a.i./L (lethal concentration; LC_1) and 150 µg a.i./L (LC_{10}). After 24 h of pyriproxyfen exposure, backswimmers were individually transferred into glass vials containing 100 mL of dechlorinated tap water and left for one hour to acclimatize. Subsequently, the number of L2 larvae preyed upon by backswimmers that were individually exposed to one of three larval densities (three, six, or nine larvae/100 mL of dechlorinated tap water) were recorded. At least seven replicates (i.e., backswimmer) were used for each combination of insecticide concentration and prey density. For each density, the larvae were transferred using a Pasteur pipette without causing injury. The number of preyed larvae (i.e., larvae carcasses floating on the water surface or in the bottom of the vial) was evaluated at 20 min intervals for two hours just after the exposure period, and the larval density re-established at each evaluation, following the Holling functional response experiment (Holling 1959; Gutiérrez et al. 2017a). All predation bioassays were performed at controlled temperature (25 ± 2 °C), humidity (60 ± 2%) and photoperiod (12 h of light phase) conditions. The naturally occurring prey deaths were not observed in our predatory assay. We checked the larval mortality at 20 min intervals for two hours, which allowed us to take care about potential larvae that naturally died during our predation bioassay. Indeed, as shown in our survival bioassays, no larval mortality was observed in the control treatments (Fig. 1A, control treatment in black lines) in the first 24 h of experiment. Regarding the predator naturally occurring mortality, we also did not observe any backswimmer death during the two hours of our predation assay. Consistent with our survival assays (Fig. 2, control treatment in black lines), backswimmer mortality does not occur within 24 hours period.

Statistical analysis

For the survival bioassay (mosquito larvae and backswimmer adults), we applied Kaplan-Meier estimators (Log-rank method) available in SigmaPlot 12.0 (Systat Software, San Jose, California, USA). We compared survival curves using the post-hoc Holm-Sidak's test. Concentration–mortality data were subjected to probit analysis (SAS Institute 2008). For the larval predation data, we used a repeated measures analysis of variance to determine the effects of insecticide, larval density, and recovery time. The number of preyed larvae in each 20 min interval on the first day after exposure were used as replicates (within-sample variation) to avoid problems associated with temporal pseudo-replication (von Ende 1993; Paine 1996). The GLM procedure with the PROFILE statement was used for this analysis using SAS software (SAS Institute 2008). Regression analysis was performed, using the curve-fitting procedure in SigmaPlot 12.0, to detect trends in predation parameters from each treatment through time. We chose the regression

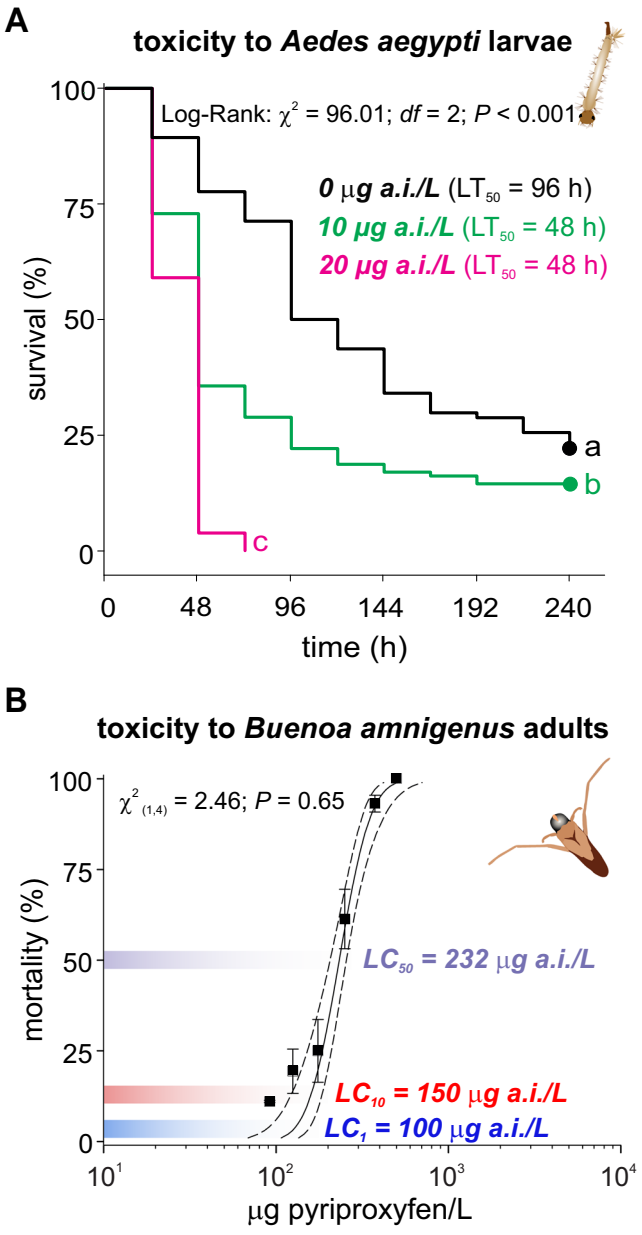

Fig. 1 Toxicity of pyriproxyfen to *Aedes aegypti* second instar (L2) larvae and backswimmer *Buenoa amnigenus* adults. A Survival curves of mosquito larvae exposed to pyriproxyfen concentrations of 10 and 20 µg a.i./L. **B** Concentration-response curve of pyriproxyfen for backswimmer adults after 24 h of exposure. The lines denote the lethal concentration (LC) values based on concentration-mortality bioassays using probit analyses. Dotted lines represent 95% confidence intervals for the LC estimations. Symbols (± standard error) show the average mortality recorded for each insecticide concentration

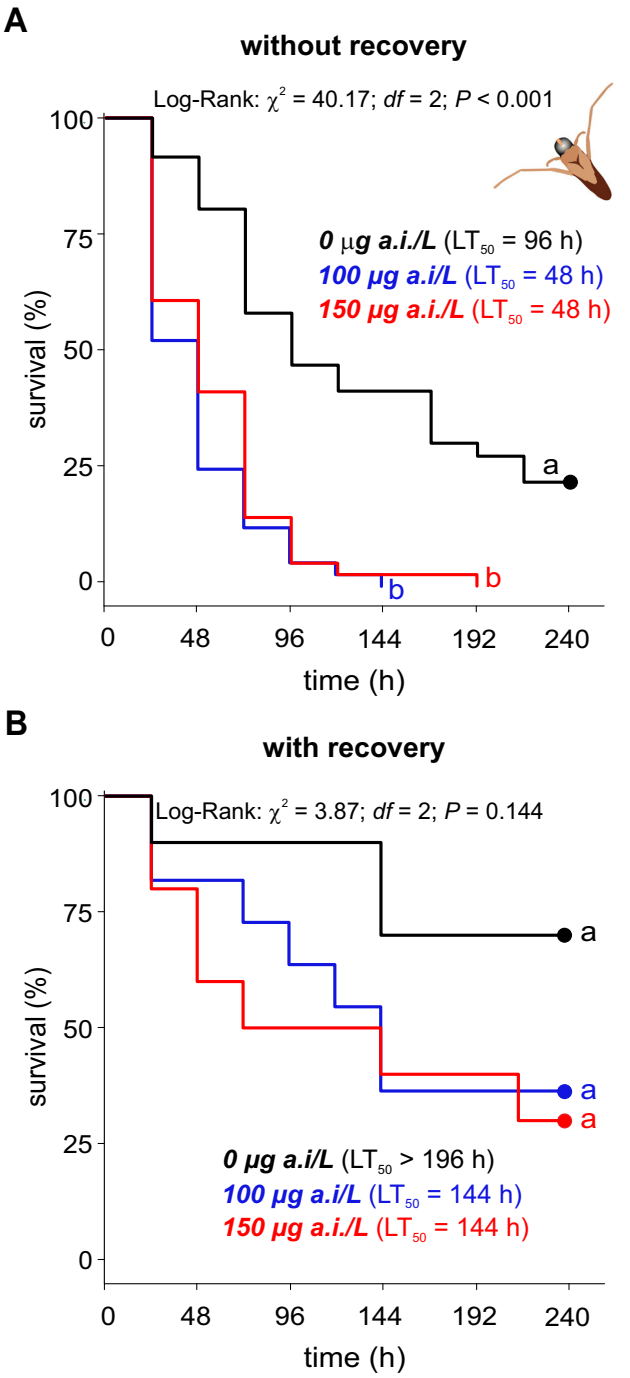

Fig. 2 Survival abilities of backswimmer *Buenoa amnigenus* adults exposed to pyriproxyfen concentrations of 100 and 150 µg a.i./L. A Survival curves of *B. amnigenus* exposed to pyriproxyfen for 10 consecutive days (without insecticide recovery). **B** Survival curves of backswimmer adults exposed to pyriproxyfen for 24 h and then transferred to insecticide-untreated water (with insecticide recovery). Treatments with the same letter do not differ according to Holm-Sidak's test ($P > 0.05$)

model based on parsimony, lower standard errors, and steep increases in R^2 with model complexity. The total number of mosquito larvae consumed was subjected to one-way analyses of variance (ANOVA) with Tukey's HSD post-hoc analysis, $P < 0.05$ (SAS Institute 2008). We assessed the assumptions of normality and homogeneity of variance using the UNIVARIATE procedure (SAS Institute 2008), and no data transformations were necessary.

Table 1 Toxicity of pyriproxyfen to backswimmers, *B amnigenus*. Lethal concentration (LC_{50}) values were estimated based on concentration-mortality bioassays using probit analysis. All concentrations are expressed in μg a.i./L

N[a]	Slope ± SE[b]	LC_1 (95% IC)[c]	LC_{10} (95% IC)[c]	LC_{50} (95%IC)[c]	DF[d]	χ^{2e}	*P*-value[f]
242	2.53 ± 0.49	100 (68–135)	150 (114–177)	232 (205–255)	4	2.46	0.65

[a]Number of insects used. [b]Standard error. [c]Confidence interval of LC_{50} at 95% probability. [d]Number of degrees of freedom. [e]Chi-square test. [f]Probability value.

Table 2 Analysis of variance with repeated measures over time for the mean number of *A. aegypti* second instar larvae (L2) preyed upon (at 20 min intervals) by *B. amnigenus* adults after 24 h of exposure to pyriproxyfen (100 or 150 μg a.i./L)

Sources of variation	df	F	*P*		
Between factors					
Insecticide (I)	2	2.06	0.1564		
Density (D)	2	0.78	0.0531		
I × D	4	1.74	0.1517		
Error	62	-	-		
	df_{num}	df_{den}	Wilks' lambda	F	*P*
Within each factor					
Time (T)	5	8	0.614	7.29	<0.0001**
T × I	10	116	0.854	0.95	0.4942
T × D	10	116	0.497	4.86	<0.0001**
T × I × D	20	193	0.523	2.08	0.0059*

Results

Toxicity of pyriproxyfen to *Aedes aegypti* larvae and *Buenoa amnigenus* adults

Survival analyses showed significant differences between the pyriproxyfen concentrations for mosquito larvae (Log-Rank: $\chi^2 = 96.01$, $df = 2$, $P < 0.001$) (Fig. 1A) and *B. amnigenus* adults (Log-Rank: $\chi^2 = 40.17$, $df = 2$, $P < 0.001$) (Fig. 2A). The median lethal time (i.e., LT_{50}) for larvae exposed to the concentration of pyriproxyfen recommended for mosquito control (i.e., 10 μg a.i./L) was 48 h. A similar median lethal time for backswimmers was observed only at concentrations as high as 100 μg a.i./L, indicating that pyriproxyfen is approximately 10-fold more toxic to mosquito larvae than to backswimmers. The concentration-mortality results for pyriproxyfen in backswimmers were satisfactorily fitted by a probit model ($\chi^2 = 2.46$; $df = 4$, $P = 0.65$), which allowed the estimation of a median lethal concentration (LC_{50}) of 232.4 (205.4–255.6) μg a.i./L (Fig. 1B, Table 1). The estimated LC_1 and LC_{10} values for backswimmers were 100.0 (68.8–135.3) and 150.0 (114.0–177.6) μg a.i./L, respectively. All backswimmer adults exposed to a single pulse of sublethal levels of pyriproxyfen (i.e., LC_1 and LC_{10}) for 24 h survived and were then transferred to dechlorinated tap water where the survival ability was assessed. Survival analyses did not show significant differences between the pyriproxyfen concentrations and control for backswimmer adults (Log-Rank: $\chi^2 = 3.87$, $df = 2$, $P = 0.144$). Backswimmers survived 3-times longer (i.e., LT = 144 h) than individuals that did not have the chance of recovery from pyriproxyfen exposure (i.e., LT = 48 h) (Fig. 2).

Effects of pyriproxyfen on the predatory abilities of *Buenoa amnigenus* adults

A repeated measure ANOVA revealed that neither pyriproxyfen exposure nor prey density affected the predatory abilities of *B. amnigenus* adults (Table 2, Fig. 3). Results, however, revealed significant effects of time ($F_{(5,6)} = 7.29$, $P < 0.0001$), the interaction between time and density ($F_{(10,116)} = 4.86$, $P < 0.0001$), as well as for the interaction between time, pyriproxyfen exposure, and density ($F_{(20,193)} = 2.08$, $P = 0.0059$). No significant effects were found for the interaction between time and pyriproxyfen exposure ($F_{(20,193)} = 2.08$, $P = 0.49$) (Table 2, Fig. 3). At the lowest prey densities (i.e., three *A. aegypti* larvae/100 mL of water), individual backswimmers that survived sublethal pyriproxyfen exposure (150 μg a.i./L) preyed upon a greater number of mosquito larvae at the first evaluation, reducing their predatory performance over time (Fig. 3A), which resulted in non-significant differences for the total number of mosquito larvae preyed upon when compared to those recorded for backswimmers not exposed to pyriproxyfen (Fig. 4A). A similar pattern was recorded for the experiment with the intermediate number of larvae (i.e., six mosquito larvae/100 mL of water), with the difference being a change recorded for the backswimmers that survived the lowest sublethal exposure to pyriproxyfen (100 μg a.i./L) (Fig. 3B). When facing the highest mosquito larvae density, the backswimmers exposed to 150 μg/L of pyriproxyfen exhibited reduced predatory abilities when compared to the unexposed insects (Fig. 3C). It is worth noting that backswimmer adults not exposed to pyriproxyfen preyed voraciously upon of mosquito larvae, eating up to 40 larvae in less than 2 h (Table 3, Fig. 4).

Discussion

We have demonstrated that *B. amnigenus* adults voraciously preyed upon mosquito larvae, especially when the prey was at higher densities. Such a finding reinforces previous

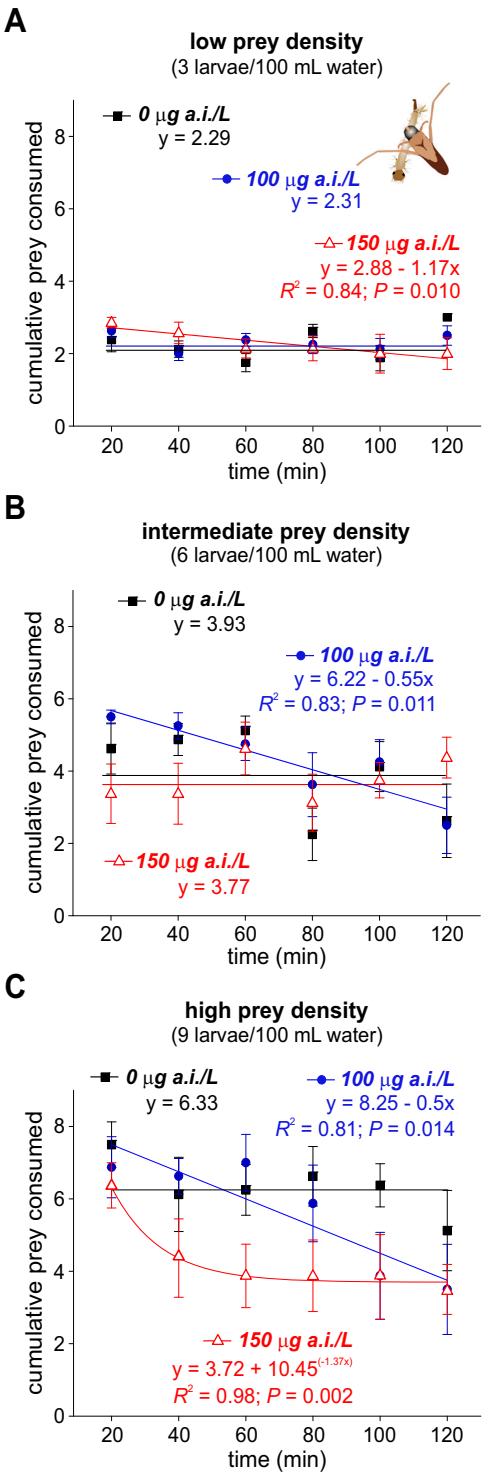

Fig. 3 Abilities of the backswimmer *Buenoa amnigenus* to prey upon *Aedes aegypti* second instar (L2) larvae. The predatory abilities of backswimmers that faced sublethal exposure to pyriproxyfen (either 100 or 150 µg a.i./L), or were unexposed, were recorded when these predators were subjected to larval densities of three (**A**), six (**B**), and nine (**C**) larvae/100 mL water. Symbols show the average number (± standard error, SE) of preyed larvae by each backswimmer

reports that make the case for backswimmers as promising biological agents to prey upon, and control, mosquito larvae populations of different species (Shaalan and Canyon 2009; Gutiérrez et al. 2017a; Eba et al. 2021). We also demonstrated that pyriproxyfen was more toxic to *A. aegypti* larvae than to backswimmers. Interestingly, our results revealed that although the abilities of adult backswimmers to prey upon mosquito larvae were slightly affected by pyriproxyfen exposure at concentrations as high as 100 µg a.i./L, the total number of preyed larvae was not reduced at the end of evaluation. Even though some studies have shown the potential of mosquito larvae to encounter their predator in artificial and natural environments (Shaalan and Canyon 2009; Sivagnaname 2009; Kweka et al. 2011; Eba et al. 2021), it is worth noting that we used *A. aegypti* larvae focusing on a prey/predator association model to test our hypothesis. Our findings, however, are likely to be applicable to other mosquito's species breeding in temporary water reservoirs or agricultural fields (e.g., rice) where a diverse aquatic insect fauna is present (Shaalan and Canyon 2009).

Although insecticide selectivity has recently been investigated in different aquatic invertebrate species (Ser and Cetin 2015; Gutiérrez et al. 2016; Santos et al. 2018; Valbon et al. 2018; Reegan et al. 2020), it remained unclear whether pyriproxyfen could impact the survival and predatory abilities of backswimmers. Our results clearly demonstrate differential susceptibilities to pyriproxyfen between the mosquito larvae and backswimmer adults, which can be explained in different ways. Firstly, given that the primary mode of action of pyriproxyfen is disrupting the insect endocrine system, maintaining the insect in an immature state until their death or reducing the number of emerged adults (Sullivan and Goh 2008; Ginjupalli and Baldwin 2013), it is reasonable to consider that the pyriproxyfen binding site receptors are more promptly available in the second instar mosquito larvae than backswimmer adults. Indeed, in a recent investigation low pyriproxyfen concentration (i.e., 2.5 µg a.i./L) were able to reduce the survival and predatory abilities of nymphs of the water bug *Belostoma anurum* (Herrich-Schäffer) (Hemiptera: Belostomatidae), a naturally occurring mosquito control agent in the Neotropical region (Valbon et al. 2019a, 2021).

Our findings may not be sufficient to rule out the possibility that *B. amnigenus* have higher tolerance to pyriproxyfen by having differential molecular interactions between pyriproxyfen and their receptor site receptors. The results described here, however, are sufficient to suggest that backswimmers' tolerance likely reflects their physiological status and life stage as demonstrated in other predator-prey relationships. For instance, differences in the properties of the

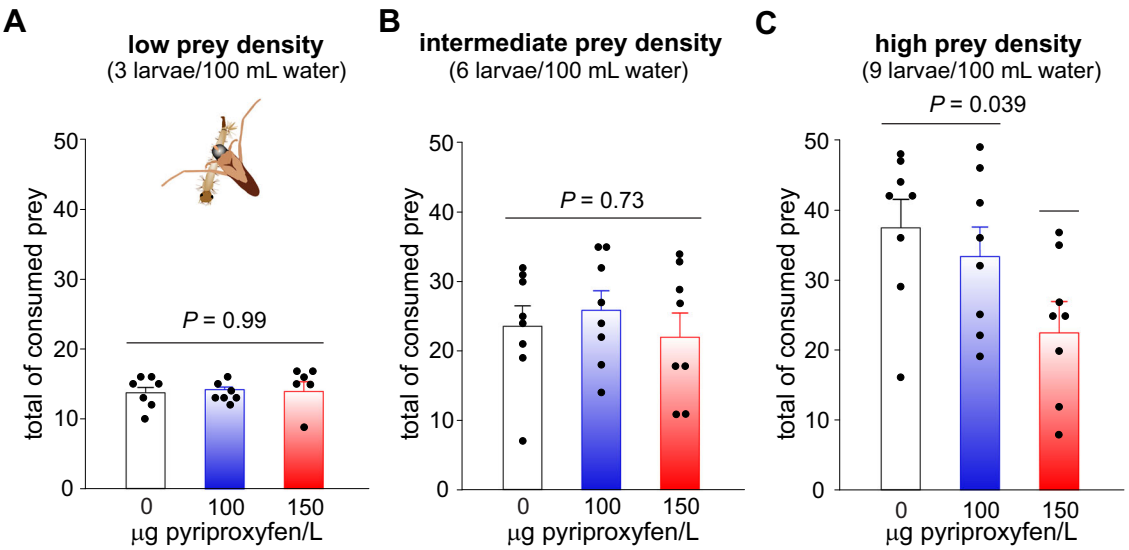

Fig. 4 The total number of *Aedes aegypti* second instar (L2) larvae preyed upon by backswimmers *Buenoa amnigenus* at the end of the experiment (2 h). The predators' abilities were assessed at larval densities of three (**A**), six (**B**) and nine (**C**) larvae/100 mL of water. The results represent the average number (± standard error, SE) and dots denote the value of each replicate. Means grouped under the same horizontal line are not significantly different by Tukey's HSD test ($P < 0.05$)

Table 3 Predation rates of *B. amnigenus* adults feeding upon *A. aegypti* second instar (L2) larvae after 24 h of exposure to pyriproxyfen (100 or 150 µg a.i./L). The results represent the average number (± standard error, SE)

Treatments	Low prey density 3 larvae/ 100 mL water	Intermediate prey density 6 larvae/ 100 mL water	High prey density 9 larvae/ 100 mL water
Control	13.75 ± 0.75a	23.63 ± 2.90a	38.00 ± 3.82a
100 µg a.i./L	13.88 ± 0.48a	25.88 ± 2.76a	33.75 ± 3.95ab
150 µg a.i./L	13.71 ± 1.52a	22.00 ± 3.50a	23.14 ± 3.83b

Means followed by different letters indicate significant differences within the same column between treatments (Tukey's HSD test, $P < 0.05$)

cuticle (e.g., thickness and binding proteins) (Wood et al., 2010) and insecticide metabolism by detoxifying enzymes (e.g., cytochrome P450 monooxygenases, glutathione-S-transferase, and general esterases) of backswimmers and mosquito larvae could also contribute to explain the pyriproxyfen selectivity as described for other aquatic insects (Liu 2015; Valbon et al. 2019b).

Since backswimmers are cosmopolitan predators, and are among the first insects to colonize freshwater habitats (Giller and McNeill 1981), they are constantly prone to reach insecticide-contaminated ecosystems in both artificial and natural water reservoirs. As notonectid adults can fly well, however (i.e., semi-aquatic organisms), they can easily evade contaminated habitats by migrating to more favorable environments (Briers and Warren 2000; McCauley and Rowe 2010). Our results for the survival abilities of *B. amnigenus* under such a scenario, i.e., facing a 24 h pyriproxyfen exposure and placed under non-contaminated environment, revealed that backswimmers did not show any difference in their survival abilities when compared to those pyriproxyfen-unexposed predators.

Recent studies have reported that mosquito larvicides can impair key behavioral parameters in aquatic predators, such as locomotion and prey catching abilities, leading to unsuccessful foraging (Gutiérrez et al. 2017b; Valbon et al. 2018; Lajmanovich et al. 2019). Interestingly, at low and intermediate prey densities, backswimmers that were previously exposed to pyriproxyfen preyed on a higher number of mosquito larvae than unexposed insects (i.e., soon after pyriproxyfen exposure). A similar foraging pattern has been reported in a coexisting backswimmer, *Buenoa tarsalis* (Truxal), when sublethally exposed to *Bacillus thuringiensis* var *israelensis* (Bti) toxins-based larvicide (Gutiérrez et al. 2017a). These observations suggest that backswimmers may share hormetic-like responses (i.e., when an unexpected beneficial effect occurs in individuals facing non-lethal stresses, (Guedes et al. 2017)) in insecticide-contaminated environments. As we found no alteration in the total number of larvae preyed upon by backswimmers exposed to sublethal levels of pyriproxyfen, at both mosquito larval densities, further studies are, however, required before drawing firm conclusions. At the highest prey density, we found that backswimmers exposed to high pyriproxyfen concentrations (150 µg a.i./L, a concentration 15-fold higher than that recommended for field application) preyed upon significantly fewer mosquito larvae than unexposed predators.

Considering the functional response of a predator, which describes the relationship between the number of prey it consumes per unit of time and the density of its prey (Holling 1959), some concerns should be taken in consideration. Here, we observed a significant effect of prey density and time in our predation bioassays. Indeed, *B. amnigenus* adults consumed more prey when exposed to higher larval density. Regarding the effect of time observed, it is worth noting that for the control (unexposed backswimmers) treatment for all prey density, we did not observe any reduction (at least for the evaluated time) in the number of consumed prey indicating that satiation might not be involved in the effect of time for pyriproxyfen-exposed individuals. Such satiation levels, however, might be appear with a longer period of evaluation for predation bioassays which requires further investigations.

Although our results suggest that pyriproxyfen, a globally employed larvicide for mosquito management in both artificial and natural water reservoirs, may not represent a potential threat for backswimmers, more studies are still required and will help to better understand the unintended effects caused by such pollutants in an ecologically realistic scenario. For instance, our predation results reveal that pyriproxyfen does not affect a critical behavior of an important predator and prey model (i.e., backswimmer *vs A. aegypti*). Taking in consideration the trophic complexities, however, further investigations aiming to evaluate whether pyriproxyfen-exposed backswimmers are more vulnerable to its predators (e.g., small vertebrates and other invertebrates), or would be a less efficient predator of mosquito larvae when compared to other predator species (e.g., nymphs of belostomatids) would contribute to understanding the impact of insecticides on aquatic community structure. For instance, South et al. (2019) suggest that by assessing relevant behaviours of both predator and prey species altogether represents more meaningful understanding of trophic complexities in an aquatic environment altered by insecticide used for mosquito control.

Conclusion

The management of mosquito vectored diseases needs to be tailored by different entomological epidemiological risk scenarios and needs to consider the multitude of factors (e.g., global trade, international travel, urbanization, water storage practices, lack of resources for intervention) that may affect its implementation (Maoz et al. 2017; Roiz et al. 2018). Given its efficiency in reducing mosquito populations at low concentrations, without impairing the abilities of backswimmers to predate upon mosquito larvae, our findings suggest that pyriproxyfen-exposed aquatic ecosystems may not offer higher risks to backswimmer populations and the suggested lack of community effectiveness following the use of pyriproxyfen to control vector transmitted diseases may lie elsewhere. Given that pyriproxyfen disrupts insect immature stages from developing into adulthood, to increase our understanding of the impact of this pesticide on both prey and predator further experiments aiming to evaluate transgenerational effects will help to produce a better insight into such sublethal effects in these naturally occurring mosquito larvae predators.

Data availability

The authors declare that the data supporting the findings of the present study are available within the article and from the corresponding author upon request.

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Author contributions WV: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. SHCA. Investigation, Formal analysis, Writing - review & editing. RSN: Investigation, Formal analysis. JFB: Identified the insect species, Writing – review & editing. PLN: Funding acquisition, Supervision, Writing - review & editing. EEO: Conceptualization, Formal analysis, Supervision, Funding acquisition, Writing - original draft, Writing - review & editing.

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Compliance with ethical standards

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