

Effect of habitat complexity on the predation of *Buenoa fuscipennis* (Heteroptera: Notonectidae) on mosquito immature stages and alternative prey

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ABSTRACT: Notonectids are well-known predators in aquatic habitats, where mosquito larvae, chironomids, and cladocerans constitute their main diet. Our purpose was to assess the effect of structural complexity on the predatory ability of *Buenoa fuscipennis*, a common predator in aquatic habitats of Buenos Aires city (Argentina). *Buenoa fuscipennis* showed type 2 functional responses in both the presence and absence of prey refuge and no differences in attack rate or handling time between refuge treatments. Regarding mosquito size classes, *B. fuscipennis* exhibited a significantly higher preference for 2nd instar larvae and no predation on pupae. In the presence of mosquito larvae and alternative prey, *B. fuscipennis* preferred mosquitoes over chironomid larvae and adult cladocerans over mosquito larvae. No switching behavior was detected in our experiments. Habitat structure only slightly affected the predator's consumption rates on mosquito larvae. Overall, preference for prey did not vary with the presence of refuge, except for the preference for mosquitoes over chironomid larvae, which was significantly decreased in the presence of refuge as a consequence of reduced predation on mosquito larvae. The results suggest that *B. fuscipennis* could efficiently control mosquitoes in structurally simple habitats where chironomids are the most abundant alternative prey but not in temporary pools where cladocerans are abundant. *Journal of Vector Ecology* 38 (2): 215-223. 2013.

Keyword Index: *Culex pipiens*, *Moina*, *Chironomus*, prey preference, functional response, switching.

INTRODUCTION

In addition to the annoyance caused by the blood-sucking habit of females, mosquitoes (Diptera: Culicidae) are vectors of several diseases. The use of appropriate methods for mosquito control is the only way in which these diseases can be prevented or controlled. The need of environmentally friendly strategies to control mosquitoes has recently increased because of growing criticisms and restrictions on the use of chemical methods (Becker et al. 2010).

The life cycle of mosquitoes includes an aquatic phase, during which immature stages develop and most of the population regulation processes occur. Predation is one of the most important biological interactions in aquatic environments, and this interaction is recognized as a major force in shaping freshwater communities (Sih et al. 1985, Wellborn et al. 1996). Predation by invertebrates is especially important in fishless ponds (Wellborn et al. 1996), and several studies have shown that community structure and variations in mosquito abundances can be explained by this interaction (Blaustein 1998, Chase and Knight 2003).

Among the attributes of predators affecting the predator-prey relationship are their consumption ability, the variation of this consumption ability at different prey densities (functional response), and the preference or selectivity for different prey sizes or types (Quiroz-Martínez and Rodríguez-Castro 2007). Some of the desirable characteristics of a predator from the point of view of mosquito control are: a) its ability to consume the target prey from early instars onwards (Ellis and Borden 1970) and its preference for advanced

instars to avoid the compensatory effect of the reduction in competitive interactions among the surviving prey (Juliano 2007); b) its preference for target prey instead of other prey types which are frequently more abundant in natural habitats (Quiroz-Martínez and Rodríguez-Castro 2007), but also its ability to feed on alternative prey when the target prey is not available or has been extinguished (Murdoch et al. 1985); c) a non-stabilizing functional response (i.e., type 2) with a high saturation density (Juliano 2007), and a high attack rate (Quiroz-Martínez and Rodríguez-Castro 2007).

Notonectids are well known to be efficient predators on mosquito larvae in ground pools (Mogi 2007). The species of this family exhibit a high searching or attack rate (Quiroz-Martínez and Rodríguez-Castro 2007) and a preference for mosquito immature stages over other prey like chironomids or cladocerans, which, nevertheless, constitute the main part of their diet in natural conditions (Mogi 2007). The consumption of these species is influenced by the combination of predator and prey size (Scott and Murdoch 1983), and by the habitat overlap between predators and prey (Mogi 2007, Quiroz-Martínez and Rodríguez-Castro 2007). Notonectids prey mainly in the water column, where they share the habitat with immature mosquitoes and cladocerans but not with other prey types like chironomids, which are more associated with the bottom of the pool (Klecka and Boukal 2012), living inside or at the surface of the substrate of aquatic habitats, where they comprise an important fraction of the macrozoobenthos.

Submerged vegetation (algae and higher terrestrial and aquatic plants) is the main component of habitat structure in

aquatic environments (Tokeshi and Arakaki 2012). Habitat structure affects interactions of mosquitoes with their predators (Juliano 2009), including the interaction of *Culex pipiens* with notonectid predators (Sih 1986). Prey detection by notonectids relies on both visual and tactile mechanisms (Peckarsky 1984, Diéguez and Gilbert 2003, Gergs et al. 2010) and is likely to be affected by behavioral changes of prey in the presence of refuge (Mogi 2007). Such behavioral changes include reduced movements and increased use of refuge in the presence of notonectid predators (Sih 1986).

Aquatic habitats in Buenos Aires city (Argentina) exhibit a great variability in the presence and abundance of vegetation in both temporary (Fischer et al. 2002) and permanent pools (Fontanarrosa et al. 2004). These habitats host several mosquito species, among which *Culex pipiens* is one of the most abundant (Fischer et al. 2000). This species is important from epidemiological and sanitary points of view, since it is a known vector of West Nile virus (Hamer et al. 2008) and Saint Louis Encephalitis virus (Mitchell et al. 1985).

The backswimmer *Buenoa fuscipennis* (Heteroptera: Notonectidae) is a common predatory aquatic insect species in Buenos Aires (Fischer et al. 2000, Fontanarrosa et al. 2004). This species is a pioneer colonizer of temporary aquatic habitats, where it temporally overlaps with the 1st and 2nd instar larvae of different mosquito species (Fischer and Schweigmann 2008). This predatory species also shares the habitat with high abundances of cladocerans belonging to the genus *Moina* and with immature stages of chironomids (Fontanarrosa et al. 2004). Thus, cladocerans and chironomids might represent potential alternative prey for *B. fuscipennis* in pools and ponds of this region.

The aim of this work was to assess the potential of *B. fuscipennis* to control mosquito immature stages in habitats of different structural complexity. To this end, we analyzed the effect of prey refuge on the preference for different larval instars and pupae, the functional response, and the preference and switching behavior among different prey types in laboratory experiments.

MATERIALS AND METHODS

Experimental methods

The predators used in this study (adult specimens of *B. fuscipennis*, total length \pm SD: 6.20 \pm 0.27 mm) were collected in local aquatic habitats two to seven days before the beginning of the experiments and fed *ad libitum* with mosquito larvae (3rd and 4th instars) until the beginning of each experiment.

The immature stages of mosquitoes were obtained from egg rafts collected in highly productive artificial containers and afterwards raised in the laboratory in plastic trays, either pooled or individually. Larvae were fed on a diet of dry yeast supplied in excess. Once the instar needed for each experiment was reached, a sample of larvae in each tray was separated and identified to species level, confirming that all corresponded to *Cx. pipiens*.

Among the alternative prey, *Chironomus* sp. larvae (Diptera: Chironomidae) were obtained from egg masses collected from the same aquatic habitat as the predators

and raised on a diet similar to the one used for mosquito larvae until the desired size was reached. *Moina* sp. adults (Cladocera: Moinidae) were collected from local temporary aquatic habitats and maintained in the laboratory on a mixed diet of dry yeast and fish food (Tetra Min[®]) until the corresponding experiment.

All experiments were performed in the laboratory, with a controlled temperature \pm SD of 27 $^{\circ}$ \pm 1 $^{\circ}$ C, in plastic trays (17 x 30 x 9 cm) containing 3 liters of tap water aged for 24 h (reaching a depth of approximately 6 cm). In each case, a treatment with and without refuge for prey was performed. Refuge treatments consisted in adding three pieces of 44 green polypropylene yarn segments (12.5 x 0.2 cm width), tied on the end to a stone to ensure submergence, simulating the presence of submerged and emerging aquatic vegetation. This would be equivalent to a density of 2,588 stems/m².

In each experiment, a single predator was confronted with different densities and/or types of prey during a predetermined time interval. Once the experiment had finished, the predator was separated and the remaining living prey were counted in each replicate. Prey death was estimated as the difference between initial and final number of prey alive. Each predator was used in one opportunity to ensure the independence of results. Two control replicates with the corresponding prey types in the absence of predators were performed to confirm that the experimental conditions were adequate for the survival of all prey types. No mortality of prey was observed in the 4-h controls. Mortality of mosquito larvae was negligible (0.25%) and identical in treatments with and without refuge in 24-h controls.

Functional response

The consumption rate on 2nd instar mosquito larvae was assessed during 24-h experiments, under a 14:10 (L:D) photoperiod. The choice of the 2nd instar was based on the results of a preliminary experiment, where a higher predation rate of *B. fuscipennis* on this instar was observed. A total of 12 replicates for each of seven prey densities (12, 18, 30, 48, 78, 125, and 200 individuals) were performed for two refuge treatments (with and without refuge).

To assess the type of functional response, the proportions of consumed prey with and without refuge were adjusted to a cubic logistic regression (Juliano 2001) of the form:

$$N_e/N_0 = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{(1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3))}$$

where N_e is the number of consumed prey, N_0 is the number of initial prey individuals, and P_0 , P_1 , P_2 , and P_3 are the parameters to be estimated by the model. These parameters were estimated using the CATMOD procedure in SAS software (SAS Institute Inc. 2009). Data of each refuge treatment were fitted individually to the equation, and types of functional responses were determined by examining the signs of P_1 and P_2 . A negative linear parameter (P_1) would indicate a type 2 functional response, while a positive linear parameter (P_1) together with a negative quadratic parameter (P_2) would indicate a type 3 functional response (Juliano

2001).

Once the type of functional response was defined, the parameters were estimated by means of a non-linear least square regression which is appropriate to situations with variable prey densities during the experiment (Juliano 2001). In the following equation, T_h (handling time) and a (attack rate) are the parameters to be estimated, and T is the total duration of the experiment (24 h).

$$N_e = N_0 (1 - \exp(a(T_h N_e - T)))$$

Pairwise comparisons of the functional response parameters obtained with and without refuge were performed with the indicator variable method (Juliano 2001) to assess the differences between refuge treatments

$$N_e = N_0 (1 - \exp(-(a + D_a(j))(T - (T_h + D_{th}(j))N_e)))$$

where j is the indicator variable that takes a value of 0 for the first data set (with refuge), and 1 for the second data set (no refuge). The parameters D_{th} and D_a estimate the difference between both data sets in the parameters T_h and a respectively, with 95% Confidence Intervals. The estimation of the parameters and the comparison with the indicator variable method were implemented with the NLIN procedure of SAS software.

Preference for mosquito instars

The experiment lasted 4 h and was performed under light conditions. In each container, a single predator was confronted simultaneously with 20 individuals of each of the following prey sizes (total length \pm SD in parentheses): L1 (1.80 \pm 0.48 mm), L2 (2.89 \pm 0.64 mm), L3 (4.36 \pm 0.57 mm), L4 (6.27 \pm 0.53 mm), and pupae (4.43 \pm 0.42 mm) of *Cx. pipiens*.

Total consumption was compared between refuge treatments with the t test for independent samples. Preference for different prey sizes was analyzed with Manly's preference index for variable prey densities (Manly 1974, Chesson 1983). This index was calculated for each prey category as:

$$\alpha_i = \ln((n_{i0} - r_i)/n_{i0}) / \sum \ln((n_{j0} - r_j)/n_{j0})$$

where n_{i0} is the number of prey of category i available at the beginning of the experiment, and r_i is the number of consumed prey of category i at the end of the experiment. Preference indexes in the prey and refuge treatments were compared with a two-way ANOVA. Post-hoc comparisons were performed by means of Tukey's test.

Preference for mosquito larvae in the presence of alternative prey

Two experiments were performed to assess the preference for mosquito larvae in the presence of two alternative prey types. In the first experiment, 2nd and 3rd instar larvae of *Cx. pipiens* (total length \pm SD: 4.13 \pm 0.23 mm) were used together with *Chironomus* sp. larvae of approximately the same size (total length \pm SD: 3.94 \pm 0.54 mm). In the second experiment,

2nd instar larvae of *Cx. pipiens* (total length \pm SD: 3.39 \pm 0.31 mm) were used together with *Moina* sp. adult individuals (total length \pm SD: 1.06 \pm 0.12 mm). The experiments lasted 4 h and were performed under light conditions. In each container, a single predator was confronted with the two prey types simultaneously. Three different relative densities of both taxa were compared, while total density remained constant. Six replicates were performed for each combination of densities (32:16, 24:24, and 16:32 individuals of each taxon) and refuge treatment (with and without refuge).

In each experiment, total consumption (mosquito larvae and alternative prey pooled) and consumption of each prey type separately were compared between refuge treatments with the t test for independent samples. Preference for mosquito larvae was assessed with Manly's preference index for variable prey densities (Manly 1974, Chesson 1983) and compared among treatments with a two-way ANOVA. Post-hoc comparisons were performed by means of Tukey's test.

RESULTS

Functional response

The number of consumed prey increased for higher prey densities in both treatments. *Buenaofuscipennis* showed a type 2 functional response in both the presence and the absence of prey refuge, which is evidenced by the negative value of the linear term in the polynomial regression of the proportion of consumed prey in relation to prey density (Table 1). A higher consumption of prey was recorded in the treatment without refuge at all prey densities (Figure 1).

The estimated attack rate was higher, and the estimated handling time was lower in the treatment without refuge than in the treatment with refuge (Table 2), although these differences were not significant according to the indicator variable method (Table 2). The estimation of pooled attack rate and handling time showed intermediate values (Table 2).

Preference for mosquito instars

Predators consumed on average 8.5 mosquito larvae in the absence of refuge and 5.8 mosquito larvae in the presence of refuge, although no statistical differences were detected between treatments ($p = 0.31$, t test). A higher consumption rate of L2 (mean: 4.4 larvae) followed by L3 (mean: 1.8 larvae) was recorded, while L1 (mean: 0.6 larvae) and L4 (mean: 0.3 larvae) were consumed less. No predation on pupae was observed during the experiment.

The results of the ANOVA showed a significant effect of prey size on the preference of *B. fuscipennis*, but no effects of refuge or the interaction of refuge and prey size (Table 3). Post-hoc comparisons among instars showed a significantly higher preference for 2nd instar larvae ($p < 0.001$). The preference for 3rd instar larvae was significantly higher than that for 1st instar larvae ($p < 0.05$) and pupae ($p < 0.05$), while no differences among the remaining categories were detected (Figure 2).

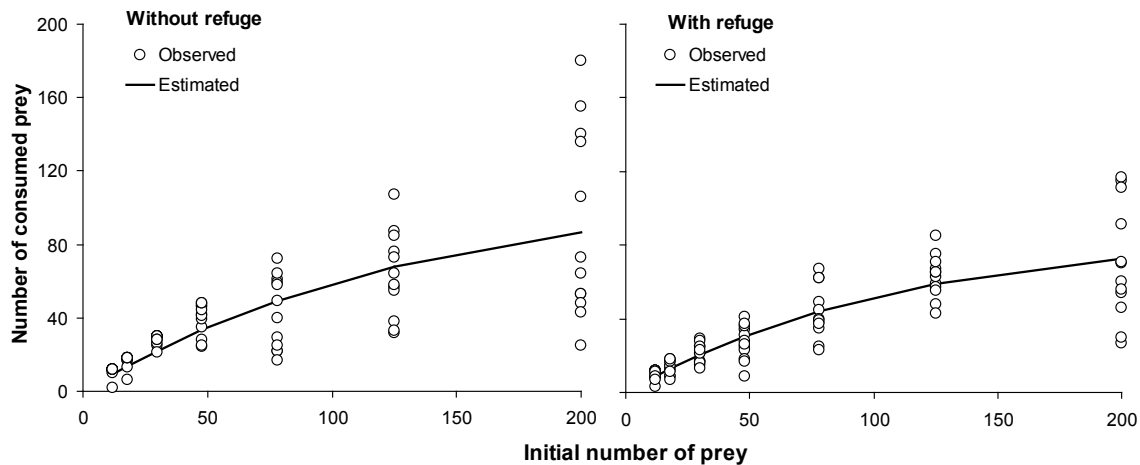


Figure 1. Functional response of *Buenoa fuscipennis* on different densities of *Culex pipiens* without refuge (left) and with refuge (right). Circles indicate observed data, and lines indicate the response predicted by the model.

Table 1. Coefficients P0 (constant), P1 (linear term), P2 (quadratic term), and P3 (cubic term) of the logistic regression of the proportion of consumed prey at different prey densities.

Parameter	Estimate	Standard Error	Chi square	P value
Without refuge				
P0	4.32832	0.3118	192.65	<0.001
P1	-0.09266	0.0105	77.81	<0.001
P2	6.55E-04	1.02E-04	41.44	<0.001
P3	-1.53E-06	2.87E-07	28.33	<0.001
With refuge				
P0	1.59213	0.1877	71.91	<0.001
P1	-0.03709	0.00726	26.11	<0.001
P2	3.06E-04	0.000076	16.33	<0.001
P3	-8.80E-07	2.23E-07	15.61	<0.001
Pooled treatments				
P0	2.5027	0.1552	260.2	<0.001
P1	-0.0518	0.00573	81.87	<0.001
P2	0.000369	0.000058	39.9	<0.001
P3	-16.13	1.69E-07	29.3	<0.001

Preference for mosquito larvae in the presence of alternative prey

The average of consumed prey in the *Culex:Chironomus* preference experiment was 15.9 prey items in the treatment without refuge, and 12.3 prey items in the treatment with refuge. No significant differences between treatments were detected in the total number of consumed prey ($p = 0.10$, t test) or in the total number of consumed chironomid larvae ($p = 0.21$, t test). On contrary, the number of consumed *Culex* larvae was significantly higher in the treatment without refuge (mean consumption: 13.4 larvae) than in the treatment with refuge (mean consumption: 8.7 larvae) ($p < 0.05$, t test).

In the *Culex:Moina* preference experiment, the average of consumed prey items was 13.7 in the absence of refuge, and 11 in the presence of refuge, and no significant differences

between refuge treatments were detected in the total number of consumed prey ($p = 0.24$, t test), in the number of consumed *Culex* larvae ($p = 0.66$), and in the number of consumed *Moina* adults ($p = 0.26$, t test).

Consumption of both prey types was recorded in all relative abundance and refuge treatments, both in the presence of *Culex* and *Chironomus* (Figure 3a) and in the presence of *Culex* and *Moina* (Figure 3b). *Buenoa fuscipennis* adults showed a greater preference for immature mosquitoes in the presence of *Chironomus* sp. larvae as alternative prey in both refuge treatments, reflected in the mosquito preference indexes higher than 0.5 (Figure 3a). In contrast, in the presence of *Moina* sp. as alternative prey, the preference for mosquito larvae in both refuge treatments was generally lower than 0.5, indicating that cladocerans were the preferred

Table 2. Functional response parameters for each treatment (individually and pooled), and comparison by the Indicator variable method. CI = confidence interval, a = attack rate, T_h = handling time in hours, Da = difference of attack rate, DT_h = difference of handling time in hours.

Parameter	Estimate	Asymptotic Standard Error	Asymptotic 95% CI
Without refuge			
a	0.066	0.019	0.028; 0.104
T_h	0.178	0.043	0.093; 0.262
With refuge			
a	0.059	0.012	0.036; 0.082
T_h	0.226	0.033	0.159; 0.292
Indicator variable method			
a	0.059	0.016	0.028; 0.090
T_h	0.226	0.044	0.138; 0.313
Da	0.007	0.022	-0.037; 0.051
DT_h	-0.048	0.057	-0.160; 0.064
Pooled treatments			
a	0.063	0.011	0.041; 0.085
T_h	0.201	0.028	0.146; 0.257

Table 3. ANOVA results of the effects of refuge treatment and prey size on preference indexes of *Buena fuscipennis*.

Factor	Sums of squares	Degrees of freedom	F	P value
Refuge	0.000	1	0.000	1.00
Prey size	4.818	4	60.396	0.000
Refuge x prey size	0.044	4	0.553	0.698
Error	0.997	50		

Table 4. ANOVA results of the effects of refuge treatment and relative prey abundances on the preference of *Buena fuscipennis* for *Culex pipiens* in the presence of two alternative prey types.

Factor	Sums of squares	Degrees of freedom	F	P value
<i>Chironomus</i> sp. larvae				
Refuge	0.258	1	8.899	0.006
Relative abundance of prey types	0.016	2	0.276	0.761
Refuge x Relative abundance	0.074	2	1.288	0.291
Error	0.870	30		
<i>Moina</i> sp. adults				
Refuge	0.014	1	0.276	0.603
Relative abundance of prey types	0.108	2	1.065	0.357
Refuge x Relative abundance	0.082	2	0.808	0.455
Error	1.518	30		

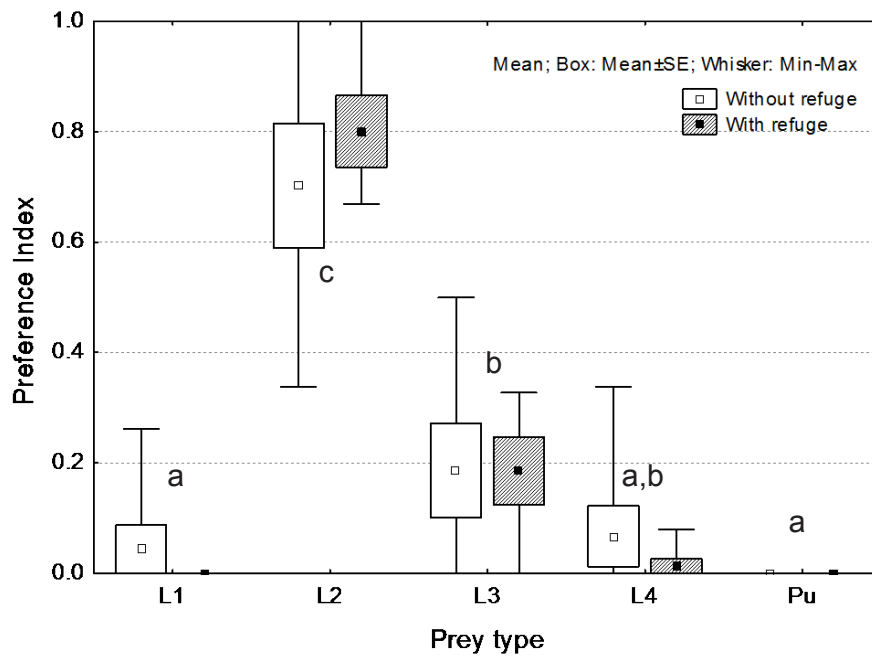


Figure 2. Preference index of *Buenoa fuscipennis* for different size categories of immature mosquitoes in two refuge treatments. Different letters indicate significant differences.

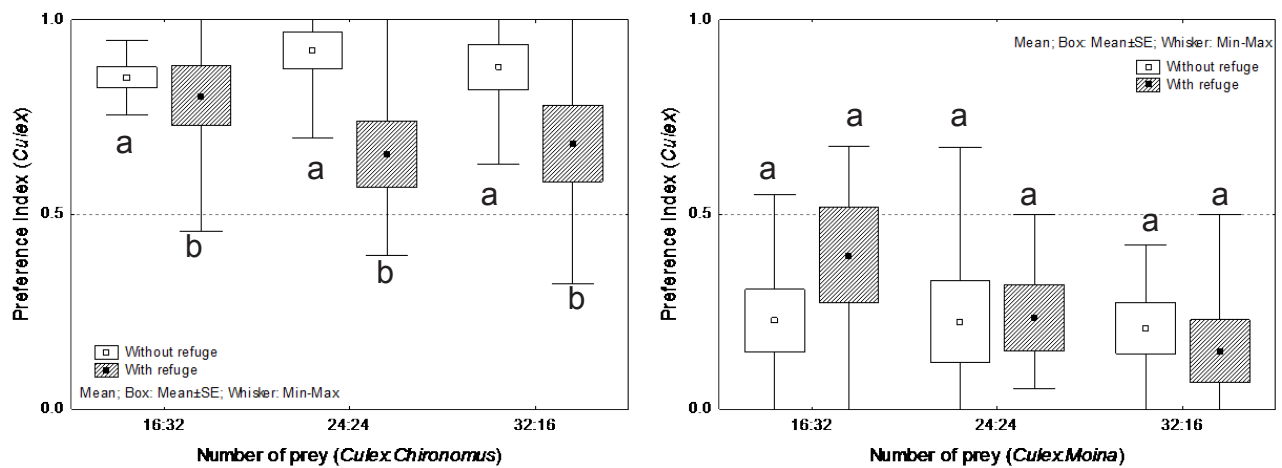


Figure 3. Preference index of *Buenoa fuscipennis* for *Culex pipiens* in two refuge treatments at different relative abundances of mosquito larvae and alternative prey a) *Chironomus* sp. larvae; b) *Moina* sp. Different letters indicate significant differences within each graph.

prey (Figure 3b).

When *Chironomus* sp. larvae were used as alternative prey, a significant effect of refuge on the preference of the predator was detected, with a lower preference for mosquito larvae in the presence of refuge. No significant effect of the relative abundance of both types of prey or of the interaction between relative abundance and refuge treatment were detected (Table 4). On the other hand, in the presence of *Moina* sp. as alternative prey, no significant effects of refuge, relative prey abundances, or the interaction term were detected on the preference of *B. fuscipennis* for *Culex pipiens* larvae (Table 4).

DISCUSSION

The results of this study show that *B. fuscipennis* is an efficient predator on *Cx. pipiens* larvae. This predator exhibited an increased consumption in the presence of higher prey densities, with a type 2 functional response in both refuge treatments. These results differ from those observed for *Notonecta glauca*, which shows a type 3 functional response in the presence of prey refuge (Hassell et al. 1977, Juliano 2001). The attack rate of *B. fuscipennis* was high and similar in the presence and absence of refuge, compared to those reported for different mosquito predators including *Notonecta irrorata* (Quiroz-Martínez and Rodríguez-Castro 2007). On the other hand, the estimated handling time suggests the capacity of this predator to consume and process between 93 and 164 2nd instar larvae of *Cx. pipiens* each day, which is approximately the number of eggs of a laying event of this species (Vinogradova 2000).

Buenoa fuscipennis showed a preference for intermediate larval instars, mainly towards the 2nd instar. In contrast, other species of the same family show a higher predation on 3rd instar larvae, e.g., *Notonecta hoffmanni* (Scott and Murdoch 1983) or *Notonecta sellata* (Fischer et al. 2012). The differences among predatory species in prey size preference could be related to differences in predator body size, since both *Notonecta* species attain larger sizes (10-12 cm length) than the species *B. fuscipennis* analyzed in the present study.

Our results show that although consumption is slightly affected, no changes in the preference for different immature stages are observed in the presence of refuge. The absence of consumption on pupae and the low preference for 4th instar larvae suggest that prey have a size refuge, which is a size above which the predator is no longer able to affect the prey. Although the body length of pupae is shorter than that of 4th instar larvae, pupae attain higher body mass (Brackenbury 2001) and have a more rounded shape, which could affect the capture success of the predator. Furthermore, escape movements of pupae are about 50% faster than those of the last larval instar (Brackenbury 2001), which could be an additional explanation for the lack of pupal consumption by *B. fuscipennis* observed in this study. It is likely that individuals that reach the size refuge benefit with an increased fitness because of the reduced intra-specific competition and the higher resource availability per individual, as a consequence of the previous reduction in population densities (Juliano

2007). On the other hand, this increase in fitness may be compensated by the non-lethal effects of the presence of notonectid predators on *Cx. pipiens* individuals, which lead to longer development times and a smaller adult size (Beketov and Liess 2007, Fischer et al. 2012). Future studies should evaluate these opposite effects, in order to assess whether these mortality factors are compensatory or additive (Juliano 2007).

The remarkable preference of *B. fuscipennis* for *Cx. pipiens* larvae over chironomid larvae in both refuge treatments is in agreement with previous observations on similar predators. For instance, *Buenoa antigone* showed a preference for *Cx. pipiens* larvae when these were offered simultaneously with *Chironomus plumosus* (Perez-Serna et al. 1996). This preference for mosquitoes remained even in those cases when various prey species were simultaneously offered to *N. undulata* (Ellis and Borden 1970) and *N. glauca* (Klecka and Boukal 2012). One possible explanation for the higher preference of *B. fuscipennis* for mosquitoes is the overlap in the microhabitat of the predator with the preferred prey, which increases the encounter probability. *Chironomus* larvae use a portion of space different from that of mosquito larvae, remaining closely associated with the substrate. They usually live in tubes, which have been associated with an anti-predator adaptation (Hershey 1987). In contrast, culicids spend most of the time hanging on the surface, breathing, or moving across the water column (Klecka and Boukal 2012), which is also the feeding habitat of notonectids belonging to the genus *Buenoa* (Quiroz-Martínez and Rodríguez-Castro 2007). Furthermore, studies on movement and speed have shown that *Cx. pipiens* larvae at least triple the maximum velocity attained by chironomids (Brackenbury 2000, Brackenbury 2001). Thus, the hypothesis that chironomids have a better escape strategy than mosquito larvae can be discarded.

On the other hand, the relatively lower preference for mosquitoes in the presence of *Moina* as alternative prey in both refuge treatments may be explained because both prey types occupy the same microhabitat in the water column, where they overlap with the predator. The preference for cladocerans in spite of their small size (even smaller than 1st instar mosquito larvae) is coincident with previous studies on *N. hoffmanni*, which has shown a higher preference for *Daphnia* sp. in the presence of *Cx. pipiens* of intermediate sizes (Chesson 1989). Previous studies have suggested that size differences are not the only explanation for prey selectivity by this notonectid predator. For instance, Scott and Murdoch (1983) concluded that the continuous movement of cladocerans vs the relative immobility of mosquito larvae would be the most likely explanation to explain the preference of this predator for the former, despite the general trend towards the consumption of larger prey.

The preference for mosquito larvae did not vary with different relative densities of prey types, suggesting that *B. fuscipennis* has no switching behavior within this range of conditions. Results of previous studies regarding the switching behavior of notonectids are controversial, since fixed preference has been reported for *Anisops bouvieri* in the presence of chironomids as alternative prey (Saha et al. 2010),

and a change in the preference for immature mosquitoes in the presence of cladocerans was observed for *N. hoffmanni*, depending on the density of each prey type (Chesson 1989). Thus, future studies should assess whether the fixed behavior of *B. fuscipennis* is maintained with more extreme relative abundances of different prey types.

Structural complexity affected the consumption of mosquito larvae by *B. fuscipennis*, indicating that the presence of refuge in the water column protected mosquitoes. Similar results have been observed previously for *N. undulata* preying on *Cx. pipiens* larvae, and have been attributed to behavioral changes of prey, that include reduced movements and the occupation of edges and protected areas (Sih 1986). Other traits of the predator, such as the type of functional response, the preference for different mosquito instars, and the preference for *Moina* sp. remained relatively constant under the conditions analyzed, suggesting that the presence of aquatic vegetation would not substantially affect the predatory behavior of *B. fuscipennis*. In contrast, in the presence of *Chironomus* sp. as alternative prey, the preference for mosquitoes was significantly reduced, mainly because of a decrease in the consumption of mosquito larvae, whereas consumption of *Chironomus* sp. remained constant. Taking into account that no refuge was provided on the substrate for chironomids, it is probable that our experimental design might have underestimated the preference for immature mosquitoes as compared to that in natural conditions, where substrate refuge for chironomids is always available. In addition, the choice of artificial refuge instead of natural aquatic plants might have affected the outcome of the experiments, and future studies should compare the effects of natural and artificial refuges on the behavior of prey and on the ability of predators to consume mosquito larvae.

The lower total consumption rate in the size preference experiment, as compared to the experiments with alternative prey, was unexpected. One possible explanation would be that the presence of not-preferred prey types, such as 1st and 4th instar larvae, and even pupae, could have been a distractive factor during the experiment, mostly taking into account that pupae and 4th instar larvae are likely to be the most easily detected prey, as observed for *N. hoffmanni* (Scott and Murdoch 1983). Taking into account that most of the behavioral explanations for our results are based on studies performed on other predatory notonectids, additional studies on *B. fuscipennis* behavior during its interaction with different prey species would largely increase the understanding of the mechanisms involved in the preference of this predator for different prey types and sizes.

The results of the present paper show that *B. fuscipennis* could exert a control on *Cx. pipiens* larvae in natural conditions, and it is likely that this predator also affects the remaining mosquito species breeding in the same habitats. Nevertheless, taking into account the differential effect on mosquitoes in the presence of different alternative prey types, it is likely that this predator is less appropriate for controlling mosquitoes in temporary environments, where the most abundant alternative prey are cladocerans, and that it will be more effective in habitats where chironomids are the

most abundant alternative prey, especially in those without abundant aquatic vegetation.

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