

Predation ability and non-consumptive effects of *Notonecta sellata* (Heteroptera: Notonectidae) on immature stages of *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT: Predators may have multiple effects on prey, including the mortality caused by consumption, but also non-consumptive effects when prey alter their life history traits in the presence of predators. This study aimed to describe the consumption ability and the non-consumptive effects of *Notonecta sellata* (Heteroptera: Notonectidae) on immature stages of *Culex pipiens* (Diptera: Culicidae). Results showed that adult *N. sellata* were capable of preying on all larval instars, although they consumed more individuals of the 2nd and 3rd instars. Immature mosquitoes raised in the presence of, but without contact with, predators showed a slower development and smaller-sized emerging adults than those raised in the control treatments. Similar survival rates were recorded in the predator and control treatments. The present study suggests that *N. sellata* adults negatively affect *Cx. pipiens* populations in two ways: a) by increasing immature stage mortality as a result of direct consumption and extended development times; and b) by reducing their number of offspring, as a result of delayed reproduction and a lower fecundity of adults. *Journal of Vector Ecology* 37 (1): 245-251. 2012.

Keyword Index: Predation, prey traits, development time, adult size.

INTRODUCTION

Culex pipiens is a widely distributed mosquito, found throughout temperate regions of the world, except in extremely cold areas (Barr 1967). This species has sanitary importance, because of its role as a vector of pathogens to humans and domestic animals (Horsfall 1955). To prevent disease transmission, vector control actions, including biological control and environmental management, have been recommended by the World Health Organization (Rejmankova et al. 1991). Recently, the interest in environmentally friendly strategies to control mosquitoes has increased, and one of the possible options is the use of different predators, including insects (Quiroz-Martínez and Rodríguez-Castro 2007, Mogi 2007, Shaalan and Canyon 2009).

Predation of different species of Notonectidae on mosquitoes has been widely documented (Mogi 2007, Shaalan and Canyon 2009), and it has been suggested that the prey-predator size combination influences selectivity between immature mosquitoes and other prey, and among different instars of mosquito larvae (Scott and Murdoch 1983).

Although the usual interest of predator-prey interaction studies has been to assess the effects of consumption on the population dynamics of prey, predators can also affect prey by causing changes in their traits (Peckarski et al. 2008). These responses of the prey are attempts to reduce the risk of predation and may be behavioral, morphological, physiological, or developmental. They can also have costs on aspects of fitness by reducing survival, development rate,

or fecundity, and are characterized as non-consumptive effects (Peacor and Werner 2008).

Mosquito immature stages exhibit short-term behavioral responses to avoid detection by predators, such as the reduction in movement and feeding rates (Juliano and Gravel 2002). These responses occur in the presence of predators, chemical cues that indicate their presence, or mechanical disturbances. The magnitude of the response to each type of signal differs among species, depending on the evolutionary history of coexistence of prey with predators (Sih 1986, Kesavaraju and Juliano 2004). Among the negative impacts of these responses on prey are reductions in foraging success or fecundity and an extension in development time with a consequent delay in the onset of reproduction (Juliano 2007).

In Buenos Aires city (temperate Argentina), *Cx. pipiens* is one of the most abundant species, and immature stages develop in a variety of breeding sites such as small (Vezzani and Albicocco 2009) and large container habitats (Fischer and Schweigmann 2010), and natural water bodies, including temporary pools (Fischer and Schweigmann 2004) and permanent ponds (Fontanarrosa et al. 2004). In natural aquatic habitats, this species frequently coexists with a variety of invertebrate predators such as coleopterans and heteropterans (Fischer and Schweigmann 2008). *Notonecta sellata* is a predatory insect that is common in temporary and permanent water bodies in Buenos Aires (Fontanarrosa et al. 2004). This species colonizes temporary habitats soon after they are filled with water following rainfalls (Fischer and Schweigmann 2008), and as such, this species has been considered a pioneer species (Bachmann and Angrisano

1998). To our knowledge, no previous studies have been performed on the predator-prey interaction of *N. sellata* with *Culex* immature stages.

The aim of the present paper was to study the predation ability of *N. sellata* on different larval instars of *Cx. pipiens* and to assess the non-consumptive effects of *N. sellata* on *Cx. pipiens*.

MATERIALS AND METHODS

Predators and prey used in this study were collected in local aquatic habitats. The predators were adult specimens of *N. sellata* (Heteroptera: Notonectidae) that were maintained in the laboratory on a diet of mosquito larvae until the beginning of the experiments. The prey consisted of individuals of *Cx. pipiens* (Diptera: Culicidae), collected as egg rafts. These were maintained in the laboratory until larval hatching, and fed with powdered baker's yeast until they reached the necessary instars. For each raft a few individuals were reared to the 4th larval instar to verify the taxonomic status of the prey.

Experimental design

The vulnerability to predation by *N. sellata* was assessed separately for each larval instar. Thirty prey individuals of the same instar were exposed to a single predator in 250 ml recipients containing 150 ml of water. The experiment lasted 24 h, after which the prey individuals that were alive were counted, and the number of killed prey was estimated by the difference. A total of 28 different predator individuals were used in seven replicates by four larval instars. The proportions of killed larvae were arcsine transformed and compared among instars by a one-way ANOVA, and post-hoc comparisons were made using the Tukey test.

To measure non-consumptive effects, larvae of *Cx. pipiens* were raised in the presence of *N. sellata* and a control treatment was performed without the predator. The experiment was conducted in 20 x 30 x 6 cm pans, containing 2 liters of dechlorinated water. Each pan contained ten individual rearing recipients, which consisted of plastic jars of 7 cm in diameter x 6 cm height, cut in the middle, with the lateral open side covered with a fine mesh screen. This design allowed the circulation of water from the pans through the jars, but prevented contact between prey and predators, thus protecting prey from being attacked. Each of ten pans was assigned randomly to treatments and two individuals of *N. sellata* were added to each predator treatment. Four rafts were used in this experiment, and a single larva of *Cx. pipiens* was transferred to each individual rearing recipient within the first 24 h of hatching. Larvae were fed daily during the first two days and every two days afterward with 0.5 mg of powdered baker's yeast diluted in 1 ml of water. Predators were fed every two days with *Culex* sp. larvae that were collected in nearby breeding sites. The experiment was carried out in the laboratory at an environmental temperature of $20 \pm 2^\circ$ C, and a constant photoperiod of 12:12 hours of light and dark. The water in the pans and in the individual rearing recipients was

maintained during the whole experiment. The survival of each prey individual and the developmental stage until the emergence of the adult were recorded every day. Individuals that reached the adult stage were sexed, and subsequently the right wing was removed from each individual and measured to the nearest 0.1 mm, using an optical microscope fitted with a graduated eye-piece. The measurements were taken from the alular notch to the distal margin excluding the fringe scales (Packer and Corbet 1989). For each pan, the survival was calculated as the number of individuals that completed the development divided by the total number of individuals at the beginning of the experiment.

The average duration of each developmental stage (four larval instars and the pupal stage), the total development time (from hatching to adult emergence), and the wing length were calculated separately for males and females in each pan. The individuals that did not complete the development were excluded from these calculations. Partial development time (four larval instars and pupal stage), total development time, and wing length were compared by means of a two-way ANOVA (treatment x sex).

To compare responses of individuals from different rafts, we calculated the mean and SD of total development time and wing length for each sex by raft, and these results were graphically analyzed. The effects of raft, treatment, and their interaction on development time and wing length were analyzed by means of two-way ANOVA. Development time data were log-transformed to meet assumptions. Post-hoc comparisons were performed with the Tukey test for unequal sample size. All statistical analyses were performed with the software Statistica version 7.1 (Statsoft, Inc. 2005).

RESULTS

Ability to consume different larval instars

The number of consumed prey varied significantly among prey instars (ANOVA $F_{4,24} = 14.64$; $p < 0.001$). More 2nd and 3rd instar larvae were consumed, although differences were not significant according to the Tukey test. A greater variability among predators was observed when preying on 4th instar larvae (Figure 1).

Non-consumptive effects

The development time analysis showed a trend towards increased times for the predatory insect treatment when compared to the control (Figure 2). This was observed for all larval instars and in the pupal stage, although the difference was significant for 4th instar larvae ($F_{1,16} = 11.17$, $p < 0.005$) and pupae only ($F_{1,16} = 6.09$, $p < 0.05$). Differences between males and females did not show a clear pattern during the 1st and 2nd larval instars, and although males developed slightly faster during the 3rd and 4th larval instars and the pupal stage (Table 1), these differences were not significant.

The complete development time lasted 11-18 days, and was, on average, shorter in the control treatment (11-15 days both for males and females) than in the predator treatment (12-17 days for males and 13-18 days for females) (Figure 2). The statistical comparison of total development

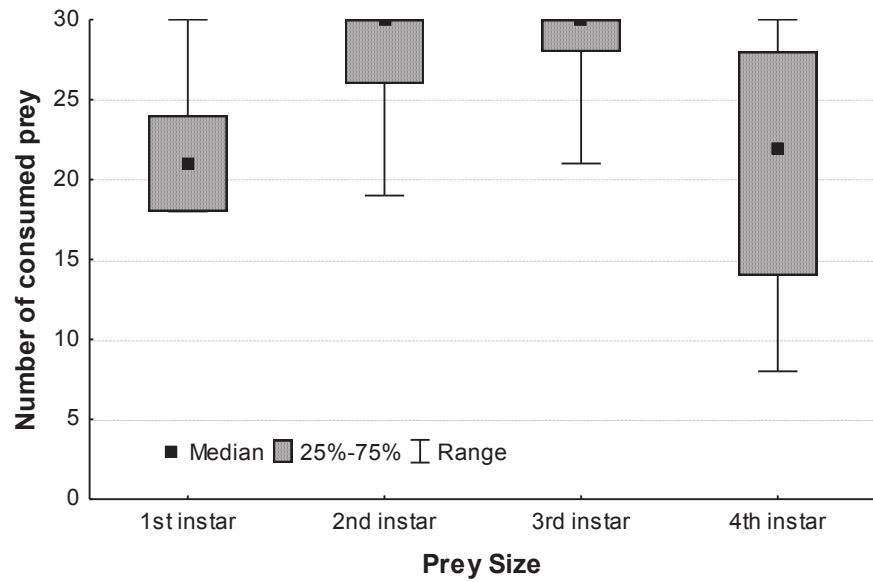


Figure 1. Box and whisker plot of the number of four larval instars consumed by *Notonecta sellata*.

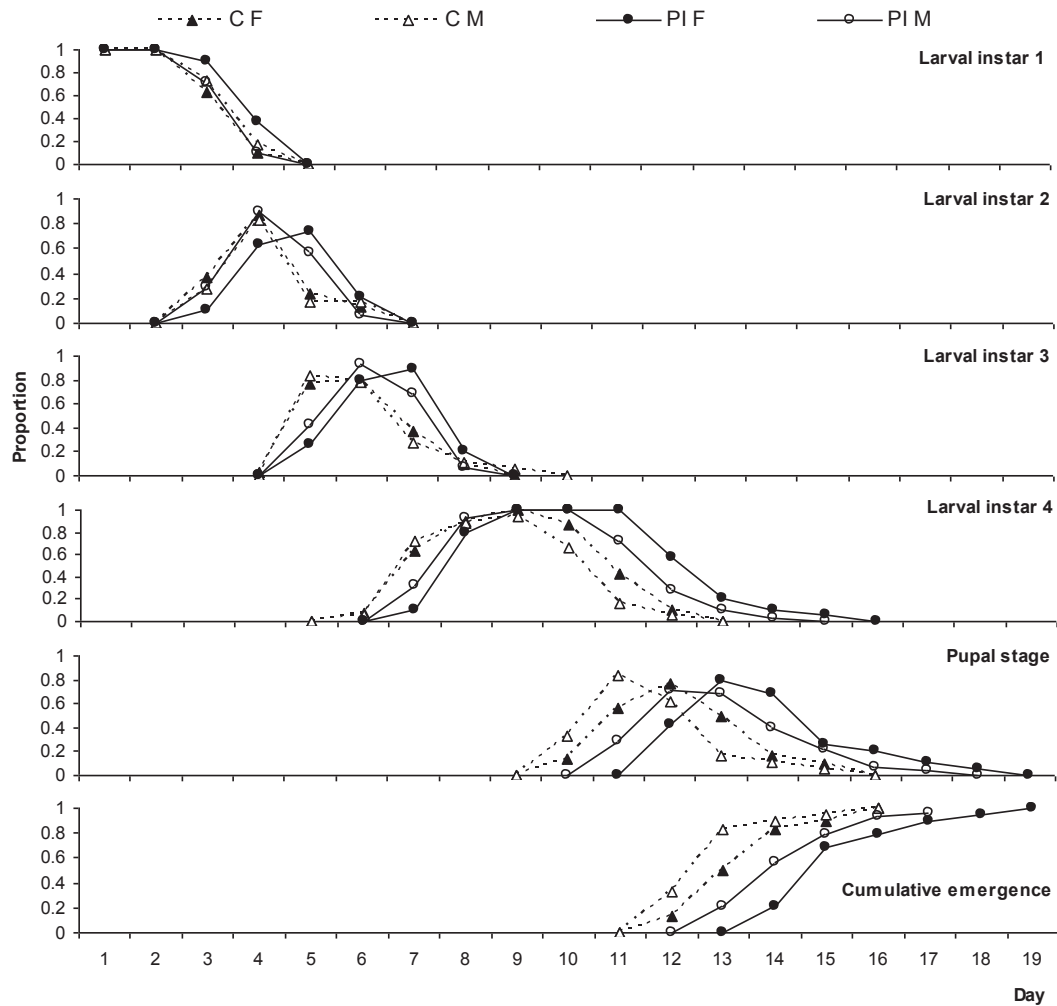


Figure 2. Temporal distribution of individuals by instars for each treatment and sex. C: Controls, PI: predatory insect treatment. F: females. M: males.

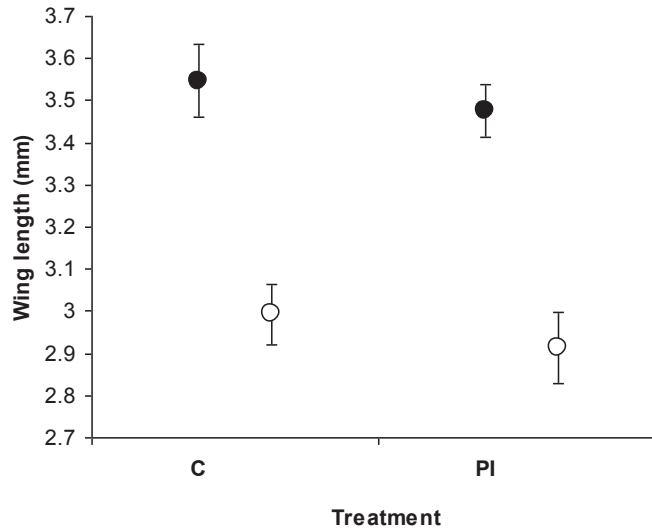


Figure 3. Mean wing length and SD by treatment and sex. Filled circles: females. Unfilled circles: males. C: Control. PI: Predatory insects.

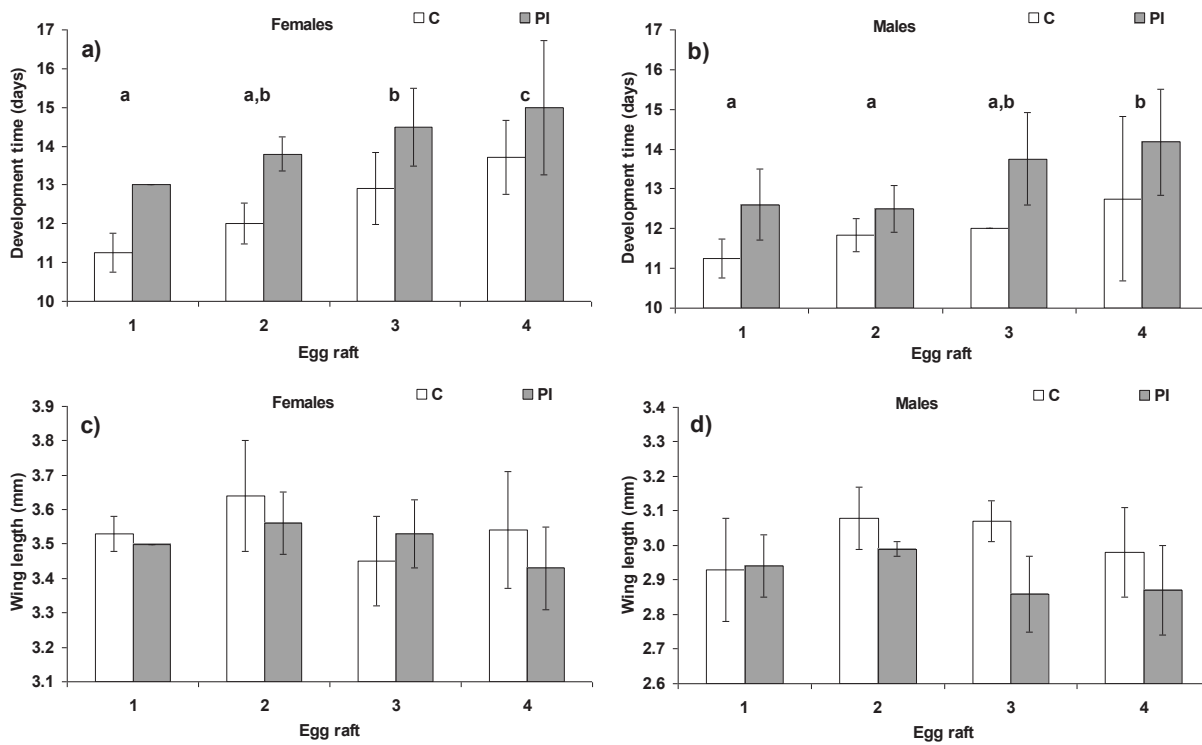


Figure 4. Mean development time (top) and wing length (bottom) for females (left) and males (right) by treatment for each egg raft. Error bars indicate SD, and different letters indicate statistically different groups.

Table 1. Mean duration in days (SD) of each developmental stage by treatment and sex combination.

	Males		Females	
	Control	P insects	Control	P insects
Larva I	2.90 (0.74)	2.95 (0.62)	2.79 (0.59)	3.12 (0.58)
Larva II	1.50 (0.50)	1.78 (0.22)	1.61 (0.41)	1.67 (0.36)
Larva III	2.00 (0.24)	2.12 (0.30)	2.08 (0.29)	2.23 (0.30)
Larva IV	3.48 (0.39)	4.35 (0.70)	3.93 (0.44)	4.90 (0.81)
Pupa	2.13 (0.30)	2.46 (0.29)	2.23 (0.22)	2.52 (0.29)

time showed significant differences between treatments ($F_{1,16} = 14.62$, $p < 0.005$), but the effect of sex ($F_{1,16} = 2.50$, $p = 0.15$) and the interaction ($F_{1,16} = 0.02$, $p = 0.88$) were not significant.

The wing length showed values between 2.70 and 3.90 mm, and was, on average, shorter for males than for females. Also, a reduction of mean wing length was observed for predatory insect treatments as compared with the control (Figure 3). Both treatment ($F_{1,16} = 4.70$, $p < 0.05$) and sex ($F_{1,16} = 259.79$, $p < 0.001$) effects were significant, but not the interaction term ($F_{1,16} = 0.02$, $p < 0.90$).

Mortality was low and homogeneous in both treatments (two individuals died in the control and three individuals in the predatory insect treatment). Four of the five dead larvae corresponded to the same egg raft (raft d).

Significant effects of egg raft on development time were detected both for females ($F_{3,41} = 7.48$, $p < 0.001$) and males ($F_{3,38} = 4.89$, $p < 0.01$). For example, both females and males of raft 1 showed the shortest development times, while females and males from raft 4 attained the longest values (Figure 4a,b). Treatment effects were significant also for females ($F_{1,41} = 20.54$, $p < 0.001$) and males ($F_{1,38} = 15.59$, $p < 0.001$), with increasing development times of all rafts in the predatory insect treatment in comparison to the control. The interaction (raft x treatment) was not significant for either sex.

Wing length showed no consistent variation of the different rafts in relation to the treatment, and statistical analyses detected no significant effects of egg raft or interaction (raft x treatment) on female and male wing length. Although a trend towards a smaller size in the presence of predatory insects was observed (Figure 4c,d), the differences were significant only for males ($F_{1,38} = 7.64$, $p < 0.01$).

DISCUSSION

Our results show the ability of *N. sellata* to consume all the larval instars of *Cx. pipiens*, which is consistent with previous observations on other predators of the same genus: *N. undulata* (Ellis and Borden 1970), and *N. hofmanni* (Scott and Murdoch 1983). The number of consumed prey is a consequence of attack rates as well as handling times, which are both known to be affected by the predator-prey size relationship. In general, attack rates show a unimodal

response to prey size for a given predator, while handling time increases for larger prey (Woodward and Warren 2007). In our experiment, the increase in the consumption rates from the first through the third larval instars might be explained by an increasing ability of the predator to detect larger prey, as it has been described for *N. hofmanni* (Scott and Murdoch 1983). On the other hand, the decreases in consumption rates of fourth larval instars are probably a result of longer handling times for each consumed prey. According to the optimal foraging theory, prey of intermediate size (i.e. second and third instars of mosquito larvae) would be the most profitable prey for this predator (Woodward and Warren 2007).

The presence of predators affected the development time and the size of adults, consistent with results obtained previously for *Cx. pipiens* under conditions of food limitation (Beketov and Liess 2007). The delay in development time and the reduction in the size of adults in the presence of predators can be explained by two different behavioral responses: a decreased feeding activity and/or an increased frequency of diving to the bottom.

Previous studies have observed a reduction in feeding activity of *Cx. pipiens* in the presence of *Notonecta undulata*, a species closely related to *N. sellata* (Sih 1986). As a consequence of reduced feeding, immature mosquitoes accumulate lower amounts of fat reserves and may exhibit a delayed molting to pupae and to adult emergence (Telang et al. 2007). On the other hand, an increase in the frequency of diving to the bottom of the recipient in the presence of predators has been documented for the pupal stage of *Cx. pipiens*. This behavior has been associated with considerable energetic costs (Rodríguez Prieto et al. 2006) and, as a consequence, the size of the adults may be reduced. Similar anti-predatory behavior has been described for the larval stage of this species as well (Kasap 1980). The longer development times of the larval and pupal stages, as well as the smaller size of the adults reared in the presence of predators, could thus be related to behavioral changes. Because this was not directly assessed in our study, we cannot distinguish whether the observed effects on life history traits are a result of a decreased energy intake (caused by lower feeding activity) or of an increased energetic cost (caused by a higher diving activity).

Independent of the causal mechanism, the trend towards longer development times in the presence of

predatory insects in our study was observed throughout the different larval instars. Differences were significant only at the fourth larval instar, which could be related to a progressive accumulation of cues indicating predation risk. These cues might be chemical signals either released by the predator alone or caused by the consumption of conspecific larvae. These two alternatives cannot be separated in the present study, but previous studies have shown that both components affect the development time of *Cx. pipiens* (Beketov and Liess 2007), suggesting the existence of additive effects. The experimental design may have underestimated the effect of the presence of predators by not exposing the experimental water to predators before the beginning of the experiment, thus limiting the accumulation of the chemical cues. Therefore, a larger effect than that observed in this study is expected in natural conditions with the continuous presence of predators.

The low and homogeneous mortality levels observed in our study suggest that this trait is the least sensitive to the risk of predation. Such results differ from a previous study that reported an increased mortality in the presence of chemical cues, indicating *N. glauca* was feeding on conspecifics. Such responses were especially evident in conditions of low food availability for larvae (Beketov and Liess 2007). Nevertheless, it should be expected that, under natural conditions, food would not be a limiting factor for *Cx. pipiens*, considering that this species has been mainly related to polluted waters with high contents of organic matter (Horsfall 1955, Campos et al. 1993).

An interesting and unexpected result is the variability in development times and adult sizes of individuals originated from different egg rafts but reared in similar conditions, which probably are a consequence of the variability in the nutritional status or genetic variability of the natural population. This should be taken into account in future studies by including a blocking effect in the experimental design.

In summary, the results suggest that the predators studied negatively affect the fitness of *Cx. pipiens* populations in two different ways: by increasing immature stage mortality and by decreasing reproductive output. Immature stage mortality is most obviously caused by direct consumption, but this effect may be increased when prey individuals remain longer in the stage exposed to consumption. Decreased reproductive output is a consequence of delayed reproduction, which implies a reduced number of yearly gonotrophic cycles of the population. Furthermore, the smaller size of adult females is related to lower fecundity (McCann et al. 2009) as well as to lower longevity (Andersson 1992), which also leads to a reduction of their offspring.

The effects on the mortality of immature stages and on reproductive output could be either additive or compensatory because of density-dependent processes (Juliano 2007). Other variables that are likely to affect the efficiency of predators are the presence of refuge (Kweka et al. 2011) and the presence of alternative prey (Jeffries 1988). These aspects should be considered in future studies in

order to accurately predict the effects of *N. sellata* on natural mosquito populations in the context of biological control.

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