



## PREDATORY EFFECT AND SELECTIVITY OF PREY OF *Notonecta peruviana* (HEMIPTERA: NOTONECTIDAE) ON THE LARVAL CONTROL OF MOSQUITOES (DIPTERA: CULICIDAE)

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**Abstract:** The larval stage of culicid mosquitoes are the main food resource of notonectides in aquatic systems. Prey alternation and abundance can significantly affect predator-prey interaction and functional response (FR). We evaluated the effect of predation and prey selectivity of *Notonecta peruviana* in fourth-stage larvae ( $F_1$ ) of *Aedes aegypti* and *Culex quinquefasciatus* at different densities and two test systems: one prey and the combination of both (ratio 1: 1). We used the FR protocol to measure predation and the Manly preference index ( $\hat{a}_i$ ) to evaluate the selectivity of prey, in a CRD experimental design. *N. peruviana* generated type II RF (“concave model”) of greater impact in larvae of *Ae. aegypti* ( $p \leq 0.01$ ). Predation capacity was similar in both of the prey,  $17 \pm 4$  larvae / day in *Cx. quinquefasciatus* and  $21 \pm 4$  in *Ae. aegypti*. The attack coefficient ( $a$ ), turned out to be similar for both prey species in both test systems and the handling time ( $Th$ ) was lower for *Ae. aegypti* than for *Culex*. *Notonecta peruviana* demonstrated selectivity for larvae of *Ae. aegypti* especially at the highest densities ( $\hat{a}_i \geq 0.5$ ), attributed to the inefficient anti depredation response, active mobility and smaller size compared to those of *Cx. quinquefasciatus*.

The FR demonstrated the success of *N. peruviana* in the larval control of culicid mosquitoes, prioritizing the type of prey; thus, promoting the need for its applicability in the field.

**Keywords:** biocontrol; predators; predatory capacity; prey consumed.

## INTRODUCTION

The larval stage of mosquitoes is an important biotic resource in freshwater ecosystems that allows measuring the magnitude of the impact of predators and offers ecologists an opportunity to identify potential controllers of medically important mosquito (Golding *et al.* 2015, Cuthbert *et al.* 2019). The *Aedes aegypti* mosquito (Diptera: Culicidae) has contributed to the spread of various diseases such as dengue, chikungunya, Zika and yellow fever in different biogeographic regions of the world; and *Culex quinquefasciatus* (Diptera: Culicidae) vector of the West Nile virus, Saint Louis encephalitis virus and lymphatic filariasis, involved in the urban transmission of the Zika virus in Brazil, the predictions of its distribution and population increase are favored by the conditions changing climatic conditions, being significantly higher (approximately 20 times) in relation to *Ae. aegypti* especially in low latitudes (Samy *et al.* 2016), increasing the risk of West Nile virus transmission in new areas such as Brazil and Peru (Ayres 2016, Samy *et al.* 2016). Both mosquitoes are distributed in America between parallels 35 ° N and 33 ° S, consistent with the latitudinal distribution of the Old World and Oceania (Lounibos 2002, Fonseca *et al.* 2006, Mills *et al.* 2010, Saul *et al.* 2017) and they can colonize natural and artificial, urban and peri-urban breeding places, important development habitats for the larvae of these organisms (Stein *et al.* 2016). In this environment, the notonectids (Insecta: Hemiptera) behave as predators of mosquito larvae, their main diet on other prey such as chironomids and cladocerans (Mogi 2007, Quiroz-Martínez and Rodríguez-Castro 2007, Fischer *et al.* 2013, Chandrasegaran *et al.* 2017). Where the complexity of the habitat, the diversity of the communities, the preference or alternation of prey and their abundance can significantly affect predator-prey interactions (Fischer *et al.* 2013, Cuthbert *et al.* 2019, Buxton *et al.* 2020a) and consequently the functional

response (FR) (Fernández-Arhex and Corley 2004). A variable that allows to evaluate the potentiality of a biocontroller, where the number of prey consumed or attacked determines the development, survival and reproduction of the predators or parasitoids (Holling 1959, Juliano 2001, Shah & Khan 2013, Li *et al.* 2018, Cuthbert *et al.* 2019). Three types of FR have been described: a linear one, with a constant growth rate (type I), a decelerating or decreasing hyperbolic rate (type II) and an increasing sigmoid relationship (type III), which vary depending on the number of prey consumed. The determination of the FR is carried out in a controlled environment and gives an idea of the entomophagous behavior and at the same time establishes comparisons between different predators (Holling 1959, Pervez 2005, Cuthbert *et al.* 2019).

The entomophagus *Notonecta peruviana* (Hemiptera: Notonectidae) is distributed in tropical and temperate zones of Peru and South America (Domínguez & Fernández 2009, Heckman 2011); several species of notonectids have been recorded as important biological controllers of culicid dipteran larvae (Fischer *et al.* 2013, Buxton *et al.* 2020b). Both mosquitoes *Ae. aegypti* and *Cx. quinquefasciatus* selected in the study show wide distribution and epidemiological importance in Peru and the Ayacucho region. *Aedes aegypti* is present in 269 districts and 18 regions (almost a third of the country) and is responsible for the transmission of the dengue virus, chikungunya, Zika and yellow fever (Espinoza *et al.* 2005, Cabezas *et al.* 2015, Requena-Zuñiga *et al.* 2016, MINSA 2019). While *Cx. quinquefasciatus* reported in urban and peri-urban areas of Peru (Lacma *et al.* 2017, Gaffigan *et al.* 2020), unlike the first species, there is no evidence of being a vector of pathogens of medical importance in Peru. Immature stages were found in larval breeding places sharing habitat with predatory species such as *N. peruviana* and several nymphal stages of odonates (Y. Ayala, personal observation).

In this context, evaluate the effect of predation

and selectivity of prey of *N. peruviana*, in the consumption of larvae of *Ae. aegypti* and *Cx. quinquefasciatus*, are fundamental for the development of robust theories and predict the success of the control measure, avoiding the escape of the target prey or it can be ignored by the controller when consuming another companion species and without epidemiological importance (Chesson 1989, Fischer *et al.* 2013, Cuthbert *et al.* 2019). In addition, it allows the development of alternative biocontrol methods (Benelli *et al.* 2016) that constitutes an alternative control based on insecticides, seriously questioned due to the resistance generated in mosquito populations and environment pollution.

## MATERIAL AND METHODS

### *Mosquito larvae*

Larvae (F<sub>1</sub>) of stage IV of *Ae. aegypti* (body size: 6 to 7 mm) and *Cx. quinquefasciatus* (7 to 9 mm), were obtained from the massive breeding developed during the months of February to April 2020 in the laboratory of Zoology of the Faculty of Biological Sciences of the National University of San Cristóbal de Huamanga (UNSCH), from of eggs collected in the field. The eggs of *Ae. aegypti* were obtained from ovitraps installed in the district of Kimbiri (La Convencion - Cusco, Peru) (12° 37'7"S; 73°47'10"W; 587 masl), according to the methods described by Fay and Eliason (1966) and Reiter *et al.* (1991). The eggs of *Cx. quinquefasciatus*, grouped in the typical way of rafts, were extracted from Reiter-type ovitraps (Reiter 1983) installed around the UNSCH campus (13° 8'45"S; 74°13'16"W; 2789 masl; Ayacucho-Peru). The eggs of both species were placed separately in plastic trays (size: 33 x 22 x 5 cm) containing 1200 mL of chlorine-free drinking water and incubated in a heated insect breeding chamber (temperature 24.5°C (± 0.5); relative humidity 65% (± 2) and a photoperiod of 12:12 (day: night). The emerged larvae were fed with crushed flakes for aquarium fish (Api Bottom Feeder Shrimp®) until they reached the pupal stage. The emerged adults were introduced to breeding cages (size: 61 x 61 x 61 cm), feeding them with a 10% sugar solution and orange slices during the first 24 hours; later,

they were fed with human blood to *Ae. aegypti* and with chicken blood to *Cx. quinquefasciatus*, until the production of eggs and to obtain after their incubation, larvae of the fourth stage of the same generation (F<sub>1</sub>) in sufficient quantity for the experimental tests (Kauffman *et al.* 2017). The fourth larval stage was used due to its physiological and morphological stability and the best attributes of biological responses that can be observed in them; the first stages are short-lived and make it difficult to assess the results reliably (Consoli & Oliveira 1994, Roberts 2014).

### *Notonectid predator*

*Notonecta peruviana* was collected in a seasonal pond with the presence of *Cx. quinquefasciatus* larvae, located in the village of Rancho, Chanchoccocha (Huamanga - Ayacucho, Peru) (13°10'50 "S; 74°15'51"W; 3,205 masl). The Notonectid adults (12 to 15 mm body length, with evident wing development and not sexed) were transported in two-liter plastic containers with a hermetic lid, containing 1500 mL of water from the sampled breeding place. At the Zoology Laboratory (UNSCH), they were kept alive in aerated aquariums containing chlorine-free drinking water and fed *ad libitum* with *Cx. quinquefasciatus* larvae up to 48 h before predation tests; 96 notonectides were individualized in disposable cups supplied with 50 mL of water to avoid cannibalism. Predator survival capacity (greater than 95%) and laying of eggs on *Elodea canadensis* plants (Alismatales: Hydrocharitaceae) were observed placed in aquariums as companion vegetation (Y. Ayala personal observation).

### *Effect of functional response on predation*

To determine the type of functional response (FR) and its implications on prey preference, two experiments were designed. The first, to test a species (prey) in six increasing densities of the fourth stage larvae of *Ae. aegypti* or *Cx. quinquefasciatus* (5, 10, 20, 35, 55 and 80 larvae / liter) for which plastic containers containing one liter of chlorine-free drinking water were used. In the second experiment, both prey species were combined in a 1: 1 ratio, with densities of 4, 8, 16, 32, 64 and 128 larvae / liter (Quiroz-Martínez *et al.* 2005). The prey densities constituted the

treatments. The larvae were introduced to the test containers and after 24 h they were used in the experiments. For each trial, a predator was incorporated, faced with the different densities and types of prey in a fixed time interval (24 h), then the number of prey consumed was counted (no mortality was evidenced in the controls). The tests were carried out with seven repetitions and their respective control for each density, prey species and their combination, under a completely randomized experimental design (CRD). The experiment was carried out under the same breeding conditions for culicid mosquitoes previously mentioned.

The logistic regression proposed by Juliano (2001) was used to distinguish the types of functional response (Type II and III), for which the number of prey consumed (expressed in proportion) was modelled as a function of the density of larvae offered in both types of trials (one species and the combination of prey), expressed in the formula:

$$Ne/N_0 = \frac{\exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}{[1 + \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)]}$$

Where  $Ne$  is the number of prey consumed,  $N_0$  is the number of initial dams,  $P_0$ ,  $P_1$ ,  $P_2$  and  $P_3$  are parameters to be estimated by the model (Fischer *et al.* 2013, Juliano 2001), calculated using the functional response test procedure of the Frail library (Pritchard *et al.* 2018) and the R Core Team (2018).

The data from each treatment were individually fitted to the equation and the types of functional response were determined by examining the signs of  $P_1$  and  $P_2$ . A negative linear parameter ( $P_1$ ) is indicative of a type II FR, while a positive linear parameter ( $P_1$ ) together with a negative quadratic parameter ( $P_2$ ) would indicate a type III FR (Juliano 2001, Fischer *et al.* 2013).

### **Prey selectivity and predation capacity of culicid mosquito larvae**

Once the type of functional response was defined in the two tests [FR Type II “concave model” proposed by Holling (1959)], the values of the attack coefficient or capture rate ( $a$ ) and average handling time ( $Th$ ) (quantitative parameters of the numerical response) necessary to determine

the predatory capacity of *N. peruviana*; For this purpose, a non-linear least squares regression was carried out, appropriate for tests with variable prey densities (Juliano 2001, Fischer *et al.* 2013) and which were evaluated based on the application of the following formula:

$$Ne = \frac{aN_0T}{1 + aN_0Th}$$

Where  $Ne$  is the number of larvae predated or consumed,  $N_0$  is the density of the dam initially offered,  $a$  is the constant attack coefficient or instantaneous search rate,  $T$  is the contact time between prey and predator (24 hours or one day), and  $Th$  is the prey handling time.

Linear regression and quantitative parameters [mean ( $\bar{X}$ ) and standard deviation (SD)] were estimated using the statistical programming language R (R Core Team 2018) and frail library (Pritchard *et al.* 2018).

Prey selectivity of *N. peruviana* in the larval control of *Ae. aegypti* and *Cx. quinquefasciatus* (two prey test system), was evaluated by comparing in pairs the number of larvae consumed ( $Ne$ ), analyzed by the Manly preference index ( $\hat{\alpha}_i$ ) for a variable population of prey, according to the type of prey tested and evaluated density (Manly 1974, Fischer *et al.* 2013), expressed in the form:

$$\hat{\alpha}_i = \frac{\ln((n_{i0} - r_i)/(n_{i0}))}{\sum_{j=1}^m \ln((n_{j0} - r_j)/(n_{j0}))}$$

Where  $n_{i0}$  is the number of prey from category  $i$  available at the beginning of the experiment,  $r_i$  represents the number of prey consumed from category  $i$  at the end of the experiment, and  $m$  is the number of prey types. The values of the Manly preference index ( $\hat{\alpha}_i$ ) vary from 0 to 1, with values for non-selective feeding of 0.5 with two types of prey in between. Index values greater than 0.5 indicate positive selection and values less to it indicate negative selection (avoidance) (Manly 1974, Fischer *et al.* 2013, Dalal *et al.* 2019a).

### **Analysis of data**

The predation capacity of *N. peruviana* was estimated by calculating the average number of larvae consumed ( $Ne$ ) for each type of prey (larvae of *Ae. aegypti* and *Cx. quinquefasciatus*) and the evaluated density. The comparison of the number

of larvae of *Ae. aegypti* and *Cx. quinquefasciatus* eaten by the predator was performed using the Mann-Whitney non-parametric test ( $\alpha = 0.05$ ) because the figures did not show a normal distribution. The results of the attack coefficient ( $a$ ) and handling time ( $Th$ ) of the entomophagous in the consumption of prey larvae exhibited normal distribution, which allowed the use of the parametric t-Student test ( $\alpha = 0.05$ ), while that the selectivity of prey was evaluated through the calculation of the Manly index ( $\hat{a}_i$ ) for each evaluated density, using the tools of the statistical program R Core Team (2018).

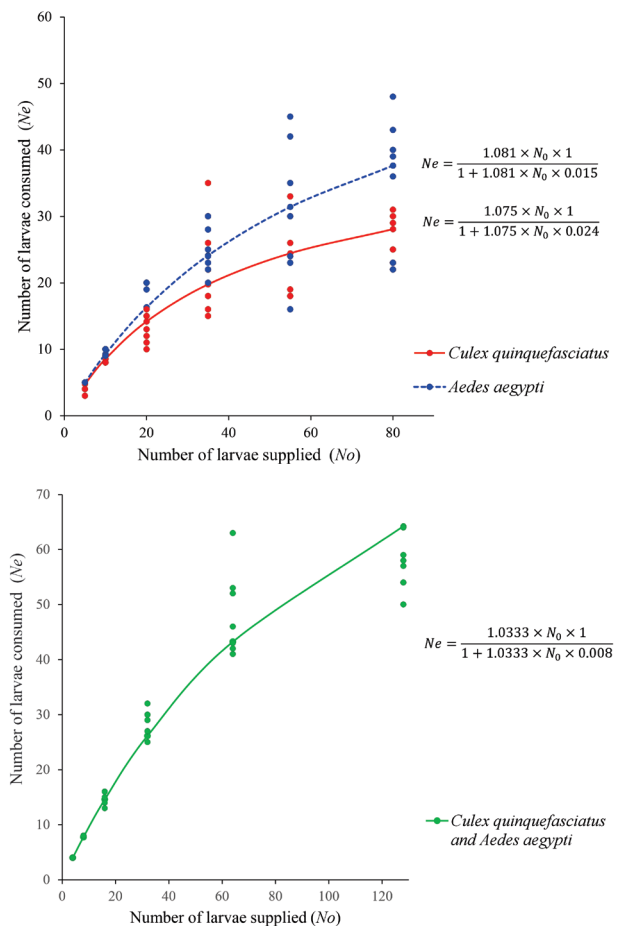
## RESULTS

### Functional response

The predation tests of *N. peruviana*, regardless of the treatments and systems evaluated (larvae of one species and the combination of prey), exhibited a type II functional response (“concave model”, Holling 1959) in the consumption of larvae of *Ae. aegypti* and *Cx. quinquefasciatus* (Figure 1: a and b), which is evidenced in the negative and significant values ( $p \leq 0.01$ ) of the estimated parameters in the logistic regression (Table 1), characterized by high attack rates at low and far lower densities to the ascending larval offerings.

### Predation capacity of culicid mosquito larvae

The results of the numerical response show that the entomophagous *N. peruviana* consumes ( $Ne$ ) an average of 4 to 23 larvae of *Cx. quinquefasciatus* ( $\pm$  SD:  $4.29 \pm 0.76$  to  $23.14 \pm 7.54$ ) and 5 to 25 larvae of *Ae. aegypti* ( $\pm$  SD:  $5.00 \pm 0.00$  to  $24.57 \pm 3.46$ ) at the lowest densities (5 to 35 larvae), stabilizing its attack at the offers of 55 to 80 larvae ( $\pm$  SD:  $24.00 \pm 6.03$  to  $26.14 \pm 3.76$  in *Cx. quinquefasciatus* and from  $30.71 \pm 10.58$  to  $35.86 \pm 9.86$  in *Ae. aegypti*), numerical values that denote subtle interest in the consumption of larvae of *Ae. aegypti* were not statistically significant ( $p > 0.05$ , Table 2), which corroborates the hyperbolic deceleration or decreasing tendency (“concave model”) of the functional response demonstrated in the experiment. In this sense, the predation of *N. peruviana* was estimated at an average of  $17 \pm 4$  larvae per day for *Cx. quinquefasciatus* and  $21 \pm 4$  larvae / day for *Ae. aegypti*.



**Figure 1.** Type II functional response of *Notonecta peruviana* in larval consumption: a) single prey test system: *Culex quinquefasciatus* or *Aedes aegypti*; b) two-prey test system: *Cx. quinquefasciatus* and *Ae. aegypti* ( $p \leq 0.01$ ), suitable for the concave model proposed by Holling (1959).

### Attack coefficient ( $a$ ) and handling time ( $Th$ )

The magnitudes of the attack coefficient ( $a$ ) tended to be similar ( $t = 0.16$ ,  $df = 6$ ,  $p = 0.881$ ) in both test systems regardless of the type of prey and larval density evaluated (Tables 3). In this concept, *N. peruviana* has an interval of  $1.075 \pm 0.110$  to locate and initiate the attack of *Cx. quinquefasciatus* larvae in a controlled environment, while for *Ae. aegypti* this coefficient was  $1.081 \pm 0.030$ ; in the combination of prey, the analyzed metric shows average values of  $1.0333 \pm 0.007$ ; CI 95%  $1.027 - 1.040$ , differences that were not significant according to the method of the evaluated variable. The estimation of the handling time ( $Th$ ) exhibited significant differences ( $t = -2.65$ ;  $df = 12$ ;  $p = 0.021$ ), demonstrating that the notonectid predator spends less time chasing, dominating, consuming

**Table 1.** Logistic regression for the type II functional response developed by *Notonecta peruviana* in the prey's test system (*Culex quinquefasciatus* or *Aedes aegypti* larvae) and the combination of preys (*Cx. quinquefasciatus* and *Ae. aegypti* larvae). \*\*\*Significance level ( $p \leq 0.01$  highly significant).

Prey larvae		Estimate	Std. Error	Value z	Pr (> z )
<i>Culex quinquefasciatus</i>	density	-0.0304	0.0024	-12.452	< 2.2e-16 ***
<i>Aedes aegypti</i>	density	-0.0375	0.0027	-13.581	< 2.2e-16 ***
<i>Culex quinquefasciatus</i> and <i>Aedes aegypti</i>	density	-0.0245	0.0014	-16.788	< 2.2e-16 ***

**Table 2.** The number of *Aedes aegypti* and *Culex quinquefasciatus* larvae consumed by *Notonecta peruviana* in the single-prey test system, at six increasing densities. P values obtained with the Mann-Whitney test for two types of prey consumed in the treatments. (No) The density of the dam was initially supplied. (Ne) Number and percentage of larvae predated or consumed.

larvae supplied (No)	larvae consumed (Ne) (mean $\pm$ SD)		W	p <sup>1</sup>
	<i>Culex quinquefasciatus</i> Ne	<i>Aedes aegypti</i> Ne		
5 (7)	4.29 $\pm$ 0.76	5.00 $\pm$ 0.00	- . -	- . -
10 (7)	9.29 $\pm$ 0.95	9.86 $\pm$ 0.38	44.5	0.33
20 (7)	13.14 $\pm$ 2.27	19.86 $\pm$ 0.38	28.0	0.002
35 (7)	23.14 $\pm$ 7.54	24.57 $\pm$ 3.46	48.0	0.609
55 (7)	24.00 $\pm$ 6.03	30.71 $\pm$ 10.58	44.0	0.307
80 (7)	26.14 $\pm$ 3.76	35.86 $\pm$ 9.86	39.5	0.11
Average larvae / day	17 $\pm$ 4	21 $\pm$ 4		

**Table 3.** Attack coefficient values ( $a$ ) and handling time ( $Th$ ) developed by the predator *Notonecta peruviana* in the consumption of *Culex quinquefasciatus* or *Aedes aegypti* larvae (one prey test system). <sup>1</sup>Level of significance with the t-Student test.

Parameters	prey	mean $\pm$ SD	CI <sub>95%</sub>	t	df	p <sup>1</sup>
Attack coefficient ( $a$ )	<i>Culex quinquefasciatus</i>	1.075 $\pm$ 0.110	0.920 - 1.170	0.16	6	0.88
	<i>Aedes aegypti</i>	1.081 $\pm$ 0.030	1.050 - 1.110			
Handling time ( $Th$ )	<i>Culex quinquefasciatus</i>	0.024 $\pm$ 0.010	0.020 - 0.030	-2.65	12	0.02
	<i>Aedes aegypti</i>	0.015 $\pm$ 0.005	0.010 - 0.020			

and digesting the larvae of *Ae. aegypti* ( $\pm$  SD: 0.015  $\pm$  0.005), getting ready for the next search and attack, compared to the higher  $Th$  required for the *Cx. quinquefasciatus* larvae ( $\pm$  SD: 0.024  $\pm$  0.010; Table 3), behavior trend also observed in the prey combination test ( $\pm$  SD: 0.008143  $\pm$  0.002; CI: 95% 0.006 – 0.009); which is to say that the notonectid predator spends less than half the time in the search, detection and consumption of *Ae. aegypti* compared to that of the larvae of *Cx. quinquefasciatus*.

### Prey selectivity of the notonectid predator

The result of the Manly test ( $\hat{a}_i$ ), exposed the entomophagus *N. peruviana* as non-selective in the consumption of mosquito larvae at

the lowest densities (4 to 16 offered prey), indistinctly devouring larvae of *Ae. aegypti* and *Cx. quinquefasciatus* ( $\hat{a}_i < 0.5$ ). Registered prey preference (positive selectivity) with increasing densities in the test medium (32 to 128 larvae), showing greater consumption of *Ae. aegypti* ( $\hat{a}_i \geq 0.5$ ) compared to those of *Cx. quinquefasciatus* (Table 4).

## DISCUSSION

Our results, regardless of the test systems (one species and the combination of prey), show that the predator *N. peruviana* develops a type II functional response (RF) ("concave model" according to Holling's proposal, Holling 1959) in

**Table 4.** Manly index ( $\hat{a}_j$ ) for the preference of *Notonecta peruviana* in the consumption of *Culex quinquefasciatus* and *Aedes aegypti* larvae in the two-prey test system (1: 1 ratio). If the index > a 0.50 for two types of prey indicate positive selection.

Dams supplied		prey	Index
No	Proportion (1:1)		
4	2:2	<i>Aedes aegypti</i>	-
		<i>Culex quinquefasciatus</i>	-
8	4:4	<i>Aedes aegypti</i>	-
		<i>Culex quinquefasciatus</i>	-
16	8:8	<i>Aedes aegypti</i>	-
		<i>Culex quinquefasciatus</i>	-
32	16:16	<i>Aedes aegypti</i>	0.5714
		<i>Culex quinquefasciatus</i>	0.4286
64	32:32	<i>Aedes aegypti</i>	0.6148
		<i>Culex quinquefasciatus</i>	0.3852
128	64:64	<i>Aedes aegypti</i>	0.5482
		<i>Culex quinquefasciatus</i>	0.4518

the consumption of larvae of *Ae. aegypti* and *Cx. quinquefasciatus*, characterized by generating destabilizing impact on the prey population and describing a high consumption ( $N_e$ ) of resources, especially at low larval densities, prey have no possibility of escape (Juliano 2001, Quiroz-Martínez & Rodríguez-Castro 2007, Dick *et al.* 2013, Fischer *et al.* 2013). Whereas when there is an excess of larvae (high densities), the entomophagous decreases its action and relaxes its predatory capacity associated to the swarm effect of the resource, the excess of prey generates confusion in the predator due to its active movement in search of the larvae and the permanent contact with them (Hassell & Varley 1969, Quiroz-Martínez & Rodríguez-Castro 2007), so the biological controller spends his time manipulating them but not consuming them (Pervez 2005).

The findings are corroborated in previous functional response analyzes reported for notonectid species preying on *Culex pipiens* larvae, *Cx. quinquefasciatus*, *Aedes aegypti* and *Anopheles breddini*, organisms in which they have a marked impact on the biotic structure in various aquatic ecosystems (Chesson 1989, Rodríguez-Castro *et al.* 2006, Blaustein *et al.* 2008, Fischer *et al.* 2012, 2013, Weterings *et al.* 2014, Cuthbert *et al.* 2019, Dalal *et al.* 2019b, Buxton *et al.* 2020a, 2020b). From this perspective, type II RF predators can cause the eradication of the species under control (e.g., larvae of *Ae. aegypti* and *Cx. quinquefasciatus*)

affecting their distribution in a wide spectrum of natural and artificial aquatic environments in urban and rural areas (Fischer *et al.* 2013, Golding *et al.* 2015, Cuthbert *et al.* 2019). Regarding the attack coefficient ( $a$ ), the results show that *N. peruviana*, regardless of the test systems, reports similar assault values for larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. However, the evidence shows that this behavior could vary depending on the type and size of prey, the development stage of the larvae and the species of predator (Fischer *et al.* 2012, 2013, Weterings *et al.* 2014, Buxton *et al.* 2020a), not detected in the test, if presumably, due to the bias originated in the experimental design when using fourth stage larvae, underestimating the other stages of development. It has been described in *Notonecta sellata* (a species related to *N. peruviana*), which can take advantage of all the larval stages of *Cx. pipiens* and show low attack coefficients (therefore greater efficiency) in the location and capture of second and third larval stage specimens, which implies according to the optimal feeding theory, that they are the most profitable prey (intermediate size prey) for developing predation tests (Woodward & Warren 2007, Fischer *et al.* 2012, Weterings *et al.* 2014, Dalal *et al.* 2019b), contrary to what was described by (Buxton *et al.* 2020a) that point to notonectides as efficient consumers of late stages of mosquito larvae and the fact that the first stages are short-lived and make it difficult to reliably appreciate

the results (Consoli & Oliveira 1994, Roberts 2014), the last criterion taken into account in the development of the research. Previous studies in species of *N. hoffmani* and *N. kirbyi* showed a reducing effect on the density of the late stage of larvae and pupae of mosquitoes and that the number of vectors was greater in the absence of these entomophages (Quiroz-Martínez & Rodríguez-Castro 2007), while the efficiency of the predators was often reduced towards the consumption of relatively large prey (Buxton *et al.* 2020a), related to the visual and tactile capacity shown by the notonectids as the main detection mechanism of the organisms to be devastated (Fischer *et al.* 2013, Chandrasegaran *et al.* 2017).

In both test systems, the handling time ( $Th$ ) registered as a general trend, a reduced unimodal response of the notonectid in the consumption of *Ae. aegypti* different to the longer time to that the larvae of *Cx. quinquefasciatus*, statistically significant and probably associated with the difference in the body size of the mosquito larvae (*Cx. quinquefasciatus* body size 7 to 9 mm and *Ae. aegypti* 6 to 7 mm) and to the greater mobility recorded in the larvae of *Ae. aegypti*. In this sense, it has been shown that large prey increase handling times ( $Th$ ) and decrease consumption rates (Woodward & Warren 2007, Weterings *et al.* 2014) and behavioral responses related to movement, that is, the behavior of the larvae writhing and resting, signify a greater threat of predation, a sign of a weak antipredation response that facilitates the entomophagus the rapid detection of the prey; contrary to what is observed in *Culex* and *Ochlerotatus* larvae that increase their resting time, decrease their feeding and movement, responses modulated according to the level of threat perceived by these organisms (Chandrasegaran *et al.* 2017). Previous studies of the predation of *N. undulata* on *Culex* versus *Aedes* larvae indicate the behavior of the larvae as the most important factor in the selection of the prey related to the escape strategies; *Aedes* larvae generally did not evade the attack of the predator, whereas *Culex* larvae did. As a result, *Aedes* larvae were attacked at much higher rates in relation to the other prey, an impact mainly associated with a recent predator-prey relationship, while the other would not be (Sih 1986, Quiroz-Martínez & Rodríguez-Castro 2007, Weterings *et al.* 2014,

Chandrasegaran *et al.* 2017). In our experience, the larvae of *Ae. aegypti* were more active, moving in the entire water column, different from that recorded in *Cx. quinquefasciatus*, whose larvae preferred to remain still on the surface of the water, which facilitates breathing, resting longer and slow down movements, increasing the escape possibilities of predators (better anti-predatory response) (Sih 1986, Brackenbury 2000, Chandrasegaran *et al.* 2017), probably being this factor and the smaller size of the larvae of *Ae. aegypti*, the most reasonable explanations for the selectivity of the predator for aedine larvae compared to those of *Cx. quinquefasciatus*, especially at the highest densities, in the Manly tests ( $\hat{a}_i$ ) for *Aedes* larvae compared to those of *Cx. quinquefasciatus*, especially at higher densities.

Our results, regardless of the test systems, demonstrated that the entomophagous insect *N. peruviana* negatively affects the larval population of *Ae. aegypti* and *Cx. quinquefasciatus*, and are consistent in generating a type II functional response ("concave model") of greater impact in larvae of *Ae. aegypti*. The predator produces a higher daily larval mortality in *Ae. aegypti* ( $21 \pm 4$  larvae / day) than in *Cx. quinquefasciatus* ( $17 \pm 4$  larvae / day), with similar attack coefficients ( $a$ ) for both species of mosquitoes in both test systems. Handling time ( $Th$ ) values were lower for *Ae. aegypti* than for *Cx. quinquefasciatus*. *Notonecta peruviana* exhibited a notable preference for the consumption of *Ae. aegypti* especially at higher densities, attributed to the inefficient anti predation response, active mobility, and smaller size compared to *Cx. quinquefasciatus* larvae, which are relatively large and of possible coevolutionary adjustment generated by a historical coexistence with the predator.

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## REFERENCES

- Ayres, C. F. J. 2016. Identification of Zika virus vectors and implications for control. *Lancet Infectious Diseases*, 16(3), 278–279. DOI: 10.1016/S1473-3099(16)00073-6
- Benelli, G., Jeffries, C. L., & Walker, T. 2016. Biological Control of Mosquito Vectors: Past, Present, and Future. *Insects*, 7(4), 52. DOI: 10.3390/insects7040052
- Blaustein, L., Kotler, B., & Ward, D. 2008. Direct and indirect effects of a predatory backswimmer (*Notonecta maculata*) on community structure of desert temporary pools. *Ecological Entomology*, 20(4), 311–318. DOI: 10.1111/j.1365-2311.1995.tb00462.x
- Brackenbury, J. 2000. Locomotory modes in the larva and pupa of *Chironomus plumosus* (Diptera, Chironomidae). *Journal of Insect Physiology*, 46(12), 1517–1527. DOI: 10.1016/S0022-1910(00)00079-2
- Buxton, M., Cuthbert, R., Dalu, T., Nyamukondiwa, C., & Wasserman, R. 2020a. Complementary impacts of heterospecific predators facilitate improved biological control of mosquito larvae. *Biological Control*, 144, 104216. DOI: 10.1016/j.biocontrol.2020.104216
- Buxton, M., Cuthbert, R. N., Dalu, T., Nyamukondiwa, C., & Wasserman, R. J. 2020b. Predator density modifies mosquito regulation in increasingly complex environments. *Pest Management Science*, 76(6), 2079–2086. DOI: 10.1002/ps.5746
- Cabezas, C., Fiestas, V., García-Mendoza, M., Palomino, M., Mamani, E., & Donaires, F. 2015. Dengue en el Perú: a un cuarto de siglo de su reemergencia. *Revista Peruana de Medicina Experimental y Salud. Pública*, 32(1), 146–156. DOI: 10.17843/rpmesp.2015.321.1587
- Chandrasegaran, K., Singh, A., Laha, M., & Quader, S. 2017. Playing it safe? Behavioural responses of mosquito larvae encountering a fish predator. *Ethology Ecology & Evolution*, 30(1), 1–18. DOI: 10.1080/03949370.2017.1313785
- Chesson, J., 1989. The effect of alternative prey on the functional response of *Notonecta hoffmani*. *Ecology* 70(5), 1227–1235. DOI: 10.2307/1938180
- Consoli, R. A. G. B., & de Oliveira, R. L. 1994. Principais mosquitos de importância sanitária no Brasil. Rio de Janeiro: Editora FIOCRUZ: p. 228.
- Cuthbert, R. N., Dalu, T., Wasserman, R. J., Callaghan, A., Weyl, O. L. F., & Dick, J. T. A. 2019. Using functional responses to quantify notonectid predatory impacts across increasingly complex environments. *Acta Oecologica*, 95, 116–119. DOI: 10.1016/j.actao.2018.11.004
- Dalal, A., Cuthbert, R., Dick, J., & Gupta, S. 2019a. Prey preferences of notonectids towards larval mosquitoes across prey ontogeny and search area. *Pest Management Science*, 76 (2):609-616. DOI: 10.1002/ps.5556
- Dalal, A., Cuthbert, R. N., Dick, J. T., & Gupta, S. 2019b. Water depth-dependent notonectid predatory impacts across larval mosquito ontogeny. *Pest Management Science*, 75(10), 2610–2617. DOI: 10.1002/ps.5368
- Dick, J., Gallagher, K., Avlijas, S., Clarke, H., Lewis, S., Leung, S., Minchin, D., Caffrey, J., Alexander, M., Maguire, C., Harrod, C., Reid, N., Haddaway, N., Farnsworth, K., Penk, M., & Ricciardi, A. 2013. Ecological impacts of an invasive predator explained and predicted by comparative functional responses. *Biological Invasions*, 15, 837–846. DOI: 10.1007/s10530-012-0332-8
- Domínguez, E., & Fernández, H. R. (Eds.). 2009. Macroinvertebrados bentónicos sudamericanos: sistemática y biología. Tucumán: Fundación Miguel Lillo: p. 654.
- Espinoza, M., Cabezas, C., & Ruiz, J. 2005. Un acercamiento al conocimiento de la fiebre Amarilla en el Perú. *Revista Peruana de Medicina Experimental y Salud. Pública*, 22(4), 308–315.
- Fay, R., & Eliason, D. 1966. A preferred oviposition site as a surveillance method for *Aedes aegypti*. *Mosquito News*, 26(4), 531–535.
- Fernández-Arhex, V., & Corley, J.C. 2004. La respuesta funcional: una revisión y guía experimental. *Ecología Austral*, 14(1), 83–93.
- Fischer, S., Pereyra, D., & Fernández, L. 2012. Predation ability and non-consumptive effects of *Notonecta sellata* (Heteroptera:

- Notonectidae) on immature stages of *Culex pipiens* (Diptera: Culicidae). *Journal of Vector Ecology*, 37(1), 245–251. DOI: 10.1111/j.1948-7134.2012.00223.x
- Fischer, S., Zanotti, G., Castro, A., Quiroga, L., & Vargas, D. 2013. Effect of habitat complexity on the predation of *Buenoa fuscipennis* (Heteroptera: Notonectidae) on mosquito immature stages and alternative prey. *Journal of Vector Ecology*, 38(2), 215–223. DOI: 10.1111/j.1948-7134.2013.12033.x
- Fonseca, D. M., Wilkerson, R. C., Smith, J. L., & Fleischer, R. C. 2006. Pathways of expansion and multiple introductions illustrated by large genetic differentiation among worldwide populations of the southern house mosquito. *American Journal of Tropical Medicine and Hygiene*, 74(2), 284–289. DOI: 10.4269/ajtmh.2006.74.284
- Gaffigan, T. V., Wilkerson, C. R., Pecor, J. E., Stoffer, J. A., & Anderson, T. 2020. Systematic Catalog of Culicidae. Walter Reed Biosystematics Unit. (Retrieved on January 25th, 2022, from <https://www.wrbu.si.edu/resources/catalog>).
- Golding, N., Nunn, M., & Purse, B. 2015. Identifying biotic interactions which drive the spatial distribution of a mosquito community. *Parasites & Vectors*, 8, 367. DOI: 10.1186/s13071-015-0915-1
- Hassell, M. P., & Varley, G. C. 1969. New inductive population model for insect parasites and its bearing on biological control. *Nature*, 223(5211), 1133–1137. DOI: 10.1038/2231133a0
- Heckman, C. W. 2011. Encyclopedia of South American aquatic insects: Hemiptera - Heteroptera: Illustrated keys to known families, genera, and species in South America. Dordrecht: Springer: p. 679.
- Holling, C. S. 1959. Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist*, 91(7), 385–398. DOI: 10.4039/Ent91385-7
- Juliano, S. A. 2001. Nonlinear curve fitting: predation and functional response curves, in: Scheiner, S.M., Gurevitch, J. (Eds.), *Design and Analysis of Ecological Experiments*. pp. 178–196. Oxford: Oxford University Press.
- Kauffman, E., Payne, A., Franke, M. A., Schmid, M. A., Harris, E., & Kramer, L. D. 2017. Rearing of *Culex* spp. and *Aedes* spp. Mosquitoes. *Bio-protocol*, 7(17), e2542. DOI: <https://doi.org/gcgzq7>
- Lacma, J. F., Iannacone, J., & Alvariano, L. 2017. Variation of entomological indicators of *Aedes aegypti* and other Culicids (Diptera: Culicidae) in two municipal parks of the province of Lima, Peru. *Neotropical Helminthology*, 11(1), 95–114. DOI: 10.24039/rnh2017111696
- Li, Y., Rall, B. C., & Kalinkat, G. 2018. Experimental duration and predator satiation levels systematically affect functional response parameters. *Oikos*, 127(4), 590–598. DOI: 10.1111/oik.04479
- Lounibos, L. P. 2002. Invasions by insect vectors of human disease. *Annual Review of Entomology*, 47, 233–266. DOI: 10.1146/annurev.ento.47.091201.145206
- Manly, B. F. J. 1974. A model for certain types of selection experiments. *Biometrics*, 30, 281–294. DOI: 10.2307/2529649
- Mills, J. N., Gage, K. L., & Khan, A. S. 2010. Potential influence of climate change on vector-borne and zoonotic diseases: A review and proposed research plan. *Environmental Health Perspectives*, 118(11), 1507–1514. DOI: 10.1289/ehp.0901389
- MINSA 2019 (Ministerio de Salud). Boletín Epidemiológico del Perú: Semana Epidemiológica 07-2019 (Volumen 28 - SE 7). Centro Nacional de Epidemiología, Prevención y Control de Enfermedades. Lima, Perú. (Retrieved on January 25th, 2022, from <https://www.dge.gob.pe/portal/docs/vigilancia/boletines/2019/07.pdf>).
- Mogi, M. 2007. Insects and other invertebrate predators. *Journal of the American Mosquito Control Association*, 23(sp. 2), 93–109. DOI: 10.2987/8756-971X(2007)23[93:IAOIP]2.0.CO;2
- Pervez, A. O. 2005. Functional responses of coccinellid predators: An illustration of a logistic approach. *Journal of Insect Physiology*, 5(5), 1–6. DOI: 10.1093/jis/5.1.5
- Pritchard, D. W., Paterson, R. A., Bovy, H. C., & Barrios-O'Neill, D. 2018. Frair: an R package for fitting and comparing consumer functional responses. *Methods in Ecology and Evolution*, 8(11), 1528–1534. DOI: 10.1111/2041-210X.12784
- Quiroz-Martínez, H., & Rodríguez-Castro, A. 2007. Aquatic insects as predators of mosquito larvae. *Journal of the American Mosquito*

- Control Association, 23(Suppl.2), 110–117. DOI: <https://doi.org/fszvbv>
- Quiroz-Martínez, H., Rodríguez-Castro, V. A., Solís-Rojas, C., & Maldonado-Blanco, M. G. 2005. Predatory capacity and prey selectivity of nymphs of the dragonfly *Pantala hymenaea*. *Journal of the American Mosquito Control Association*, 21(3), 328–330. DOI: <https://doi.org/bgdgfn>
- R Core Team. 2018. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2630 pp.
- Reiter, P. 1983. A portable battery-powered trap for collecting gravid *Culex* mosquitoes. *Mosquito News*, 43(4), 496–498.
- Reiter, P., Amador, M. A., & Colon, N. 1991. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. *Journal of the American Mosquito Control Association*, 7(1), 52–55.
- Requena-Zuñiga, E., Mendoza-Urbe, L., & Guevara-Saravia, M. 2016. Nuevas áreas de distribución de *Aedes aegypti* en Perú. *Revista Peruana de Medicina Experimental y Salud Pública*, 33(1), 171–172. DOI: [10.17843/rpmpesp.2016.331.1804](https://doi.org/10.17843/rpmpesp.2016.331.1804)
- Roberts, D. 2014. Mosquito larvae change their feeding behavior in response to kairomones from some predators. *Journal of Medical Entomology*, 51(2), 368–374. DOI: [10.1603/ME13129](https://doi.org/10.1603/ME13129)
- Rodríguez-Castro, V. A., Quiroz-Martínez, H., Solís-Rojas, C., & Tejada, L. O. 2006. Mass rearing and egg release of *Buenoa scimitra* as biocontrol of larval *Culex quinquefasciatus*. *Journal of the American Mosquito Control Association*, 22(1), 123–125. DOI: <https://doi.org/b68tjn>
- Samy, A. M., Elaagip, A. H., Kenawy, M. A., Ayres, C. F. J., Peterson, A. T., & Soliman, D. E. 2016. Climate change influences on the global potential distribution of the mosquito *Culex quinquefasciatus*, vector of West Nile Virus and Lymphatic Filariasis. *PLOS ONE* 11(10), e0163863. DOI: [10.1371/journal.pone.0163863](https://doi.org/10.1371/journal.pone.0163863)
- Saul, W. C., Roy, H. E., Booy, O., Carnevali, L., Chen, H. J., Genovesi, P., Harrower, C. A., Hulme, P. E., Pagad, S., Pergl, J., & Jeschke, J. M. 2017. Assessing patterns in introduction pathways of alien species by linking major invasion data bases. *Journal of Applied Ecology*, 54(2), 657–669. DOI: [10.5061/dryad.m93f6](https://doi.org/10.5061/dryad.m93f6)
- Shah, M. A., & Khan, A. A. 2013. Functional response - a function of predator and prey species. *The Bioscan*, 8(3), 751–758.
- Sih, A. 1986. Antipredator responses and the perception of danger by mosquito larvae. *Ecology*, 67(2), 434–441. DOI: [10.2307/1938587](https://doi.org/10.2307/1938587)
- Stein, M., Rossi, G. C., & Almirón, W. R. 2016. Sección 3: Ecología. Cap. 4. Distribución geográfica de Culicidae de Argentina. In: Berón, C. M., Campos, R. E., Gleiser, R. M., Díaz-Nieto, L. M., Salomón, O. D., & Schweigmann, N. (Eds.), *Investigaciones sobre mosquitos de Argentina*. pp. 47–56. Mar del Plata: Universidad Nacional de Mar del Plata.
- Weterings, R., Vetter, K. C., & Umponstira, C. 2014. Factors influencing the predation rates of *Anisops breddini* (Hemiptera: Notonectidae) feeding on mosquito larvae. *Journal of Entomological and Acarological Research*, 46(1), 107–111. DOI: [10.4081/jear.2014.4036](https://doi.org/10.4081/jear.2014.4036)
- Woodward, G., & Warren, P. 2007. Body size and predatory interactions in freshwaters: Scaling from individuals to communities. In: Hildrew, A.G., Raffaelli, D.G., & Edmonds-Brown, R. (Eds.), *Body Size: The Structure and Function of Aquatic Ecosystems*. pp. 98–117. New York: Cambridge University Press. DOI: [10.1017/CBO9780511611223.007](https://doi.org/10.1017/CBO9780511611223.007)

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