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Influence of density-dependent aphid consumption on the body-weight of a predaceous ladybird, *Coccinella transversalis*

Ahmad Pervez and Rupali Sharma

Abstract
We investigated the influence of density-dependent aphid consumption on the body-weight of adult male and female ladybirds, *Coccinella transversalis* Fabricius when fed on aphid, *Hysteroneura setariae* (Thomas). This density-dependent prey consumption in terms of functional response seems to modulate the body-weight of adult ladybirds, which has a direct implication on the fate of aphid biocontrol. Hence, adult male and female ladybirds were fed on aphids at varying densities (10 to 200 aphids) and the magnitude of aphids consumed and their influence on the adult body-weight were recorded. The prey consumption increased with an increase in aphid density and the aphid consumption pattern exhibits a Type II response. This simultaneously affected the body-weights of adult male and female, *C. transversalis*, which increased from 0.59 ± 0.08 to 2.72 ± 0.35 mg, and 0.69 ± 0.083 to 3.46 ± 0.37 mg, respectively, when fed on aphids at an increasing density from 10 to 200 aphids. Among adults, the female was a more potent aphid-biocontrol agent, as it possesses a greater attack rate (0.8423 ± 0.18) and can handle prey in a lesser time (0.1558 ± 0.02 hour or 9.35 ± 1.20 min), as compared to that of a male ladybird. The density-dependent aphid consumption may directly affect the body-weight, as consumption of a more significant number of aphids may increase adult bodyweights, thereby making them better biocontrol agents in terms of quantitative aphid consumption. It is concluded that adult female *C. transversalis* possesses the potential for the biocontrol of *H. setariae*.

Keywords: *Coccinella transversalis*, ladybirds, predator, functional response, *Hysteroneura setariae,* Coccinellidae

Introduction
The density-dependent prey consumption can be best described, as a functional response, which is an analytical representation of a predator's response toward an increasing prey density (Holling 1959) [7]. This response could be linear (type I), curvilinear (type II), sigmoidal (type III) (Holling 1959, 1965) [7], dome-shaped (type IV) (Köhnke *et al.* 2020) [12] or negatively exponential (type V) (Watt 1959) [30] to prey density. It explains prey-predator dynamics, predator's stability, and predictability of biocontrol success. A majority of predators, including aphidophagous ladybirds (Coleoptera: Coccinellidae) exhibit type II response (Osman and Bayoumy 2011; Omkar and Pervez 2011) [20, 17], which indicates that although prey consumption by the predator increases with an increase in prey-density, its rate of prey consumption, as the time progresses, decrease. Very few ladybirds exhibit a type III response, where prey consumption exponentially increases with prey density and thereby, these ladybirds had more significant biocontrol potential (Seyfollahi *et al.* 2019) [27]. Nevertheless, certain predaceous ladybird species with type II responses successfully suppressed aphid populations (Madadi *et al.* 2011) [14]. Multiple factors modulate this response by predaceous ladybirds like predator stage, predator mass, prey type, arena-size (Uiterwaal and de Long 2018), body-size (Chaudhary *et al.* 2015), host plant (Jalali and Ziaaddini 2017) [9], temperature (Jalali *et al.* 2010) [8], prey distribution and predator digestion (Feng *et al.* 2018) [6].

Coccinella transversalis Fabricius is an aphidophagous ladybird with a wide prey-range (Omkar and Pervez 2004a) [17]. Its augmentation could be optimized by exposing it to different mating combinations (Michaud *et al.* 2013) [15] and it co-exists with other ladybird-species with little threat to its developing immature stages (Omkar *et al.* 2005).

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It synergistically declined the population of aphid, *Aphis gossypii* (Glover), when released with ladybird, *Propylea dissecta* (Mulsant) (Omkar and Pervez 2011) [19]. Recently, we investigated how the intraspecific competition for food in terms of numerical response affected the bodyweight of *C. transversalis* (Pervez and Sharma, 2021) [23]. However, limited knowledge exists on its density-dependent prey consumption, handling time and attack rate. An increase in aphid density may lead to increased aphid-consumption during its predatory stages. We hypothesized that this increase in prey consumption would positively modulate its body-weight, which could enhance its biocontrol potential. Thus, we designed laboratory experiments using rusty plum aphid, *Hysteroneura setariae* (Thomas), which is also an important pest of many crops, *viz.* rice, wheat, sugar cane, maize and soyabean crops (Kale *et al*. 2020) [11]. The objectives of this study were (i) to determine how the adult male and female, *C. transversalis* respond to the increasing densities of aphid, *H. setariae*, (ii) the influence of this density-dependent prey consumption on their body-weight, and (iii) the impact of this prey consumption dependent body-weight on the fate of the aphid-biocontrol programme.

Materials and Methods
Stock Maintenance
Adults of *C. transversalis* were collected from the agricultural fields adjacent to the city of Kashipur, India (30.2937°N, 79.5603°E), preying on aphid, *H. setariae* infesting on creeping bluegrass, *Bothriochloa insculpta* (Hochst.). They were brought to the laboratory and paired in Petri dishes (9.0 diameter x 2.0 cm height) containing *ad libitum H. setariae* infested on host plant twigs. The females mated and laid eggs in clusters that were transferred to other Petri dishes (size as above). These Petri dishes were then kept in the Environmental Test Chamber (ETC) (*REMI, Remi Instruments*) maintained at 25±1 °C, 65±5% R.H and 12L: 12D. After egg-hatch, the first instars were shifted into 1000 ml beakers (*Borosil*) containing aphid-infested twigs (aphid and host plant as above). Each beaker contained five first-instars reared on H. setariae until adult emergence. The aphid and host plant twigs were replenished daily to avoid any contamination. The newly eclosed F_1 adults were sexed and isolated in separate Petri dishes (same size as above) and reared on the above aphid-host plant complex before being used for the experiment.

Experimental Design
12-hour starved 15-day-old unmated adult male (as predator) was weighed (W_1) using an electronic balance (*SHIMADZU*, Model ATX-224, 0.1 mg precision) and kept in a 1000ml beaker (as above) containing 10 third-instar nymph of *H. setariae* (N_o) along with a small host plant twig. A moist filter paper was kept in the beaker to maintain humidity. The beaker was covered with a muslin cloth fastened with a rubber band and then transferred to ETC (abiotic conditions as above). After three hours, this beaker was taken out and the live aphids were counted to determine the number of aphids consumed (N_a). The predator was again weighed (W_2) to estimate its weight gain ($W_e = W_2-W_1$). Using a new predator, the experiment was repeated at aphid densities (No) of 15, 25, 50, 100, 150 and 200. The experiment was replicated ten times (n=10). The entire experiment was repeated using a 12-hour starved 15-day-old virgin adult female. The initial and final body weights were subjected to a two-sampled t-Test

and the means were compared by Bonferroni's Method using statistical software, SAS 9.0 (2002). The gain in body weight was subjected to Two-way ANOVA, considering "prey density" and "sex" as independent variables and "gain in body weight" as a dependent variable using SAS 9.0 (2002).

Functional Response Analysis
We used a logistic regression model (1) before fitting the data to Holling's disc equation (Holling, 1959) [7], which determines the functional response shape by considering the proportion of prey eaten (N_a/N_o) as a function of prey offered (N_o) (Juliano 2001) [10]. It also differentiates between the Type II and Type III responses. Thus, we fitted the data to the below-mentioned model (equation-1) using the CATMOD Procedure (Juliano 2001) [10] of SAS on the statistical software SAS 9.0 (SAS, 2002).

$$\frac{N_a}{N_o} = \frac{\exp (P_o + P_1 N_o + P_2 N_o^2 + P_3 N_o^3)}{1 + \exp (P_o + P_1 N_o + P_2 N_o^2 + P_3 N_o^3)} \qquad [1]$$

Where

P_o, P_1, P_2 and P_3 are the intercept, linear, quadratic and cubic coefficients, respectively. These were estimated using the maximum likelihood method on SAS 9.0. If $P_1 > 0$ and $P_2 < 0$, the proportion of prey consumed is positively density-dependent, confirming a Type III functional response. If $P_1 < 0$, the balance of prey consumed declines monotonically with the initial number of prey offered, describing a type II response (Juliano 2001) [10]. Because the logistic regression analysis indicated a type II response for our data, further analysis was restricted. Coefficient of attack rate and Prey handling time estimated using Roger's random predator equation (Rogers 1972):

$$N_a = N_o \{1- \exp [(a)] T_h N_a - T]\} \qquad [2]$$

Where 'a' is the coefficient of attack rate, 'T' is the duration of exposure, and 'This is the handling time. This equation overcomes the problem of prey depletion (Holling 1959) [7]. We fitted the data to this equation (2) using a non-linear least-squares method (PROC NLIN Method) on SAS 9.0 (Juliano 2001) [10]. The PROC NLIN Method allows programming steps to be incorporated into the procedure, making it possible to use Newton's iterative method to solve the implicit functions (with N_a on both sides of expression) given in equation (2).

Results
Prey consumption by the adult female and male *C. transversalis* increased curvilinearly with an increase in density of *H. setariae* exhibiting Type II functional response, as also estimated by the negative values of linear (P_1) coefficients (Figure 1). The adult male and female *C. transversalis* consumed 8.1±0.99 to 44.9±5.21 and 7.4±0.89 to 37.1±4.43 aphids, respectively with an increase in prey density from 10 to 200 aphids. Significant values of maximum likelihood estimates were obtained by CATMOD Procedure (SAS) (Table 1). This confirmed the values of handling time (T_h) and coefficient of attack rate (a) obtained by the NLIN Procedure of SAS. The female had a greater attack rate and lesser handling time than those of adult male (Table-2). Two-way ANOVA revealed significant main effects of 'prey density' (F = 23.27; *P*<0.0001; d.f. = 6) and 'sex' (F = 11.31; *P*<0.001; d. f. = 1) on the gain in adult body-

weight. The interaction between 'prey density' and 'sex' was not statistically significant (F = 0.47; P = 0.83; d. f. = 6). The increase in body weight was significantly greater at higher aphid density than those at lower prey densities (Table-3) exhibiting similarity with the functional response curves (Figures 1 - 2).

Discussion

Despite the density-dependent prey consumption by adult *C. transversalis* increased with prey density, Type II functional response occurred indicating a gradual decline in the prey consumption rate thereby reaching an asymptote at high aphid abundance, as also reported on other ladybird species (Lee and Kang 2004; Pervez and Omkar 2005; Cabral *et al*. 2009; Bayoumy 2011) [2, 13, 16]. Prey handling time is a crucial parameter that determines the efficiency of a predator, as it describes the total time spent in catching, subduing, killing, and digesting the prey. At the same time, the attack rate demonstrates its aggressiveness and prey-capture ability within a time frame (Pervez and Omkar 2003) [22]. We found adult females of *C. transversalis* had a faster h. They attack aphids with a doubly attack rate compared to those of adult malesf malest male, indicating that females were better biocontrol agents. Numerous studies advocated a high attack rate and less handling time for successful biocontrol agents, *viz. Harmonia axyridis* (Pallas) (Lee and Kang 2004) [13], *Coccinella undecimpunctata* Linnaeus (Cabral *et al*. 2009) [2], and *Nephus includens* (Kirsch) (Bayoumy 2011). Contrarily, adult female *Hippodamia variegata* (Goeze) attacked aphid, *Aphis fabae* Scopoli will lesser attack rate and more handling time than adult male (Farhadi *et al*. 2010) [5]. We found that female *C. transversalis* consumed a greater number of aphids than the male, which agrees with the findings on *P. dissecta* (Omkar and Pervez 2004b) [18]. However, it disagrees with Cabral *et al*. (2009) [2], who reported a similar amount of prey consumed by an adult male and female ladybirds, *C. undecimpunctata*, and stated that it might be due to differences in the prey instars with varying body sizes. The female *C. transversalis* was more voracious than the male in terms of attack rate possibly due to her bigger body-size and greater energy requirements for egg production.

We allowed adult *C. transversalis* to consume aphids for only 3-hours to overcome the constraints due to digestion time. Papanikolaou *et al*. (2014) [21] supported such short-duration studies to provide better estimates of handling time and attack rate due to digestion constraints and other distractions if the exposure time is 24 hours. They found a significant decrease in the handling time of both male and female ladybirds, *Propylea quatuordecimpunctata* (Linnaeus) when exposed to the aphid, *A. fabae* for 3-hours as compared to a 24-hour exposure. The predatory efficiency of ladybirds likely declines with every prey they consume with time, which further supports short-termed studies for the authentic estimation of functional response parameters.

We found that the increase in aphid consumption at aphid abundance further ledincreasede in body weight. Significant main effects of 'prey density' and 'sex' on the gain in body-weight of *C. transversalis* revealed that both these independent variables modulate the adult body-weight. It was evident as adults exposed to aphid abundance were heavier than those at aphid scarcity. This indicates that the density-dependent aphid consumption modulates body-weight. Dixon (2007) [4] stated that aphid density is an important determinant of body size in aphidophagous coccinellids. Sloggett (2008) [28] reviewed bthe ody size and diets of aphidophagous ladybirds and concluded that the evolution of their body size is a function of both aphid density and prey-size. It is likely that successful aphidophagous ladybirds, *viz. H. axyridis*, *Coccinella septempunctata* Linnaeus and *Coleomegilla maculata* (de Geer) are better evolved in terms of body size, as they are more exposed to aphid abundance in the field and have dominated the coccinellid fauna compared to their co-occurring species.

It is concluded that both male and female, *C. transversalis* exhibited Type II response exhibiting negative density-dependence prey mortality. The adult females were better predators to handle prey with a greater attack rate. The density-dependent prey consumption modulates the increase in predator's body-weight, which directly associates with the fate of biocontrol programmes, as heavier adults may consume more aphids. Thus, it is predicted that female *C. transversalis* could be effective for the biocontrol of aphid, *H. setariae*.
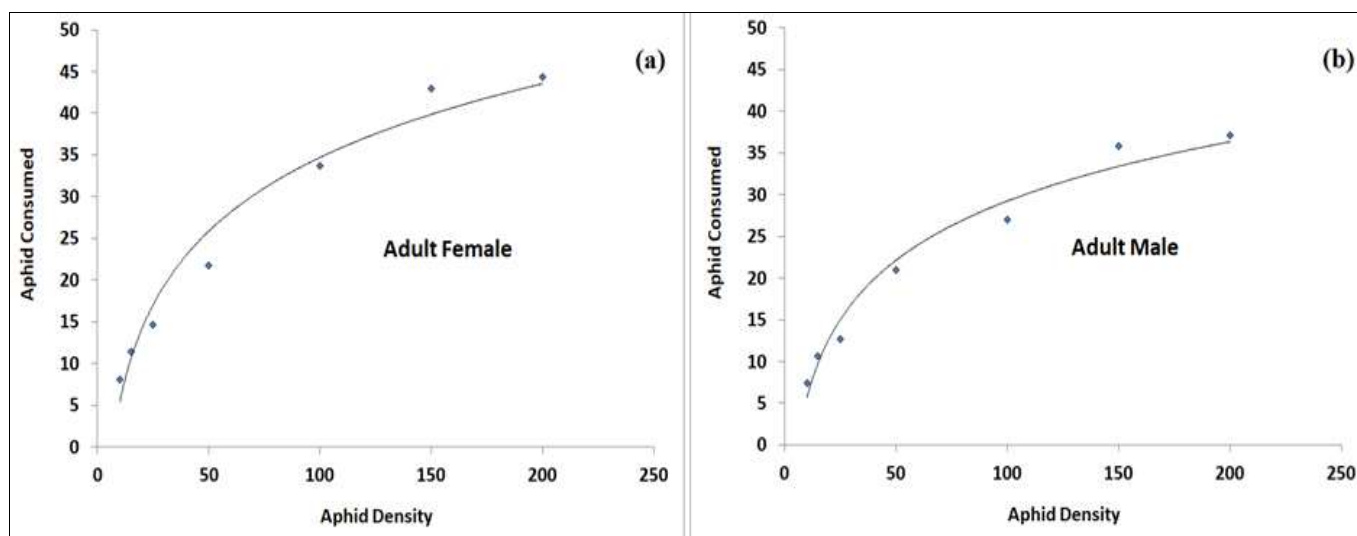

Fig 1: Functional response of adult female (a) and male (b) ladybird, *C. transversalis* against increase in density of aphid, *H. setariae*.
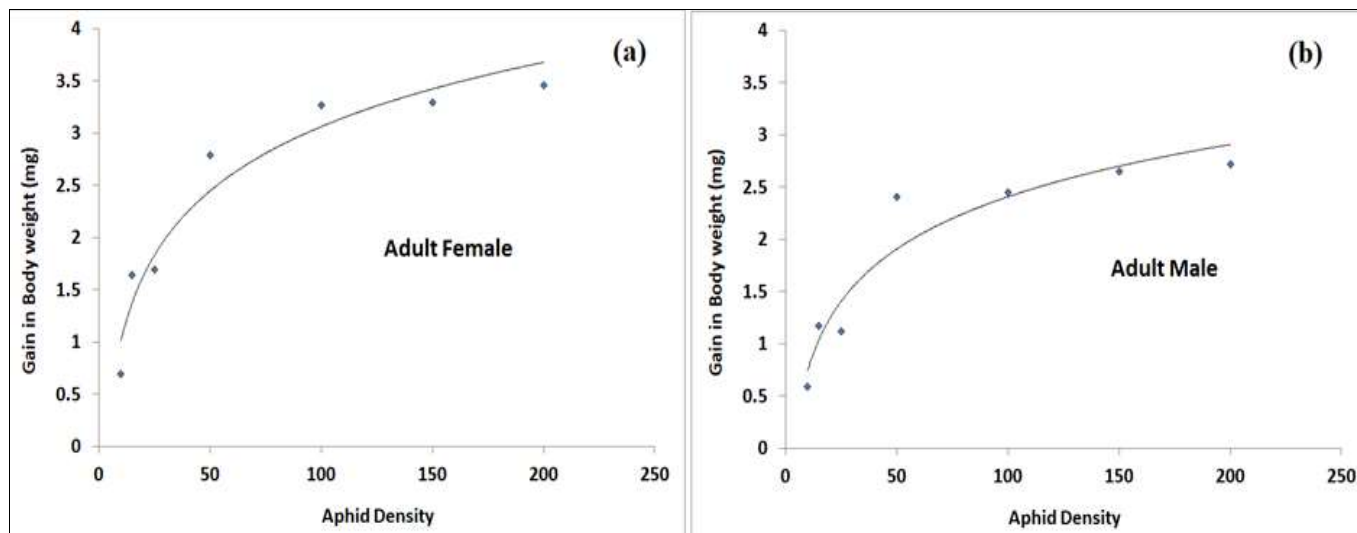
Fig 2: Relationship between the gain in the body weight of adult female (a) and male (b) ladybird *C. transversalis* and increasing density of aphid, *H. setariae*.

Table 1: Maximum likelihood estimates from Logistic Regression of proportion of prey eaten as a function of initial prey densities by adult males and females of *C. transversalis* (n=10) using PROC - CATMOD Method of SAS.

Ladybird Sex	Parameters	Estimate	S.E.	Chi Square Value	P-value
Male	Intercept (P_o)	2.3469	0.199	138.22	*P*<0.00001
	Linear (P_1)	-0.0747	0.007	99.25	*P*<0.00001
	Quadratic (P_2)	0.000538	0.000075	51.42	*P*<0.00001
	Cubic (P_3)	0.000000013	2.1E-09	36.51	*P*<0.00001
Female	Intercept (P_o)	1.5363	0.179	93.45	*P*<0.00001
	Linear (P_1)	-0.0518	0.0069	56.59	*P*<0.00001
	Quadratic (P_2)	0.000387	0.00007	30.85	*P*<0.00001
	Cubic (P_3)	0.00000009	0.000000002	36.51	*P*<0.00001

Table 2: Coefficient of attack rate (a) and handling time (Th) of adult male and female, *C. transversalis* when fed on *H. setariae* estimated from the PROC-NLIN Method of SAS.

Ladybird sex	a	Standard Error	T_h (in min)	Standard Error (in hour)
Male	0.4046	0.08	11.23 min	0.02
			0.1871 hour	
Female	0.8423	0.18	9.35 min	0.02
			0.1558 hour	

Table 3: Initial and Final weights of adult female and male *C. transversalis* when fed on *H. setariae* at varying densities

Prey density	Adult Female			Adult Male		
	Initial body weight (w_1)	Final body weight (w_2)	t-value and probability	Initial body weight (w_1)	Final body weight (w_2)	t-value and probability
10	18.12±2.10a	18.81±2.17a	t = -0.40; P=0.69	15.26±1.57a	15.85±1.63a	t = -0.52; P=0.61
15	18.14±1.92a	19.78±2.04a	t = -1.34; P=0.19	14.55±1.51a	15.72±1.62a	t = -1.72; P= 0.22
25	17.89±1.75a	19.58±1.98b	t = -2.54; *P*<0.05	12.23±1.20a	13.35±1.32a	t = -1.78; P= 0.09
50	17.9±2.02a	20.69±2.23a	t = -1.74; P= 0.10	13.75±1.43a	16.16±1.76b	t = -2.25; *P*<0.05
100	20.13±2.03a	23.4±2.34b	t = -3.13; *P*<0.01	14.6±1.52a	17.05±1.76b	t = -2.49; *P*<0.05
150	19.31±1.94a	22.6±2.22b	t = -2.42; *P*<0.05	12.98±1.32a	15.63±1.57b	t = -3.62; *P*<0.01
200	19.08±1.99a	22.54±2.34b	t = -2.64; *P*<0.01	13.89±1.40a	16.61±1.70b	t = -3.62; *P*<0.01

Data are Mean ± S.E.
Different letters within the same sex in the row mean that data is significantly different.

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