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Density and stage-specific functional response of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) on the larvae of *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae)

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Abstract

Epilachna vigintioctopunctata is a voracious pest of several economically important agricultural crops worldwide, but relatively few studies have focused on the feasibility of its predators in controlling Epilachna beetles. This study investigated the predatory efficiency and functional response of *Coccinella septempunctata* to varying densities of *E. vigintioctopunctata*. Our laboratory experiments revealed that larval and adult *C. septempunctata* attacked and preyed on Epilachna larvae and showed Type II functional response except when they were at the 1st instar stage. Based on three key parameters in the functional response, the 4th instar of *C. septempunctata* was found most efficient as a predator. The mean prey consumption increased with increasing prey density and growth stage of *C. septempunctata*. Predatory efficiency did not differ significantly among 4th instar, adult females, and males. However, the feeding percentage was inversely related to the density and higher at low prey densities. Our study concluded that 4th instar larvae and adults of *C. septempunctata* were most influential in suppressing *E. vigintioctopunctata* and this ladybird beetle could help reduce pesticide use and protect agroecological environments.

Keywords: Alternative prey, biological control, natural enemies, predator

Introduction

Epilachna beetle (Often known as Hadda beetle) *Epilachna vigintioctopunctata* (= *Henosepilachna vigintioctopunctata*) (Coleoptera: Coccinellidae) is an economically significant phytophagous pest attacking crops of Solanaceae, Cucurbitaceae, Fabaceae, Convolvulaceae, and Malvaceae and is widespread in Asia [31]. It is also known as an alien agricultural pest in Australia and South America [30]. This pest causes skeletonization of the leaves due to infestation, eventually resulting in crop yield loss and quality reduction [32, 48]. Synthetic pesticides have been the most common method to suppress the Epilachna beetles, and chemical control appears to be mostly effective in managing this pest [23, 60, 63]. However, frequent, and indiscriminate use of pesticides should negatively affect the native ecosystem and beneficial organisms such as pollinators and natural enemies [32, 48]. Therefore, incorporation of the other eco-friendly control measures is ideal for managing the pest population and conserving biodiversity for sustainable crop production [49].

Natural enemies such as parasitoids and predators commonly play a significant role in suppressing agricultural pest populations [7, 15]. Literature available suggests that this is also the case for Epilachna beetles. For example, immature stages of the Epilachna beetles are attacked and killed by several species of parasitoid wasps such as *Pediobius foveolatus*, *Pleurotropis epilachnae*, *Tetrastichus ovulorum*, *Achrysocharis appannai*, *Uga menoni* and *Bracon* sp. [30, 61 71, 72]. These studies suggest that parasitoids may be useful in controlling this pest. However, to our knowledge, very few studies have focused on the potential role of predators. There are a few records of predators of Epilachna beetles, i.e., pentatomid bug *Eocanthecona furcellata* [35] and reduviid bug *Rhynocoris fuscipes* [61]. Since native predator complexes can also be very important as the natural enemies [11] and they are often abundant in agricultural fields, studies should be done to examine the significance of predators in suppressing Epilachna beetles. Hence, there is a critical need for studying native predators to identify their potential preying

Epilachna beetles and to devise integrated pest management strategies incorporating native natural enemies to reduce pesticide use.

Coccinellid beetles are generally recognized as effective and important natural enemies of many economically deleterious pests in a wide range of natural and agricultural habitats [37, 49]. The seven-spot ladybird *Coccinella septempunctata* (Linnaeus) (Coleoptera: Coccinellidae) is widely distributed in Asia and is known as an economically important generalist predator. This predatory beetle is usually recognized as an aphid natural enemy, and aphidophagous responses of *C. septempunctata* have already been examined in several previous studies [49]. However, *C. septempunctata* also predate a range of different prey types other than aphids, such as mealybugs [3, 64], whiteflies [2], mites [38, 46], thrips [12], lepidopteran pests [1, 41], stink bugs [67], and psyllids [29], suggesting that this ladybird predator may also prey on Epilachna. However, no previous studies have examined whether *C. septempunctata* uses Epilachna beetle as their prey item. It is therefore not known whether *C. septempunctata* has potential as the biological control agent.

The predatory potential is the basic factor regulating the population dynamics of the predator-prey system [17, 50, 58]. A quantitative study of function response and searching efficiency is the essential starting point for revealing the predatory potential of a given predator species, and can thus help better understanding of predator-prey interactions and the significance of the predator impacting the prey population [34, 40, 58] because such a study allows determining the relationship between prey density and the rate of prey consumption of a predator [5, 8, 14, 43, 54, 76], the mechanism of stability, and co-evolutionary relationships to predict the potential of natural enemies as biological control candidates [6, 8, 17, 76]. Several studies with *C. septempunctata* have been conducted to determine its functional response on aphid prey [20, 76] and some non-aphid pests; bollworms [1], *Spodoptera frugiperda* [41], spider mite *Tetranychus urticae* [42], and whitefly *Trialeurodes vaporariorum* [2].

No information is available on the functional response of *C. septempunctata* on Epilachna pests. In addition, few studies have paid attention to stage-specific responses by the predator whereas the response can reasonably depend on the developmental stage of *C. septempunctata*. Hence, the current study aimed to evaluate the predation efficiency of all larval stages (1st, 2nd, 3rd, and 4th instar) and adults (female and male) of *C. septempunctata* on Epilachna, a prey previously unrecorded. Accordingly, we conducted laboratory experiments to examine the functional response to *Epilachna vigintioctopunctata* and assessed the potentiality as a biological control agent.

Materials and methods

Coccinella septempunctata and *Epilachna vigintioctopunctata* were collected from the field in Fukuoka City and were reared in the laboratory of Insect Natural Enemy, Bioresource and Bioenvironment Science, Faculty of Agriculture, Kyushu University, Japan. Cotton aphid *Aphis gossypii* (Hemiptera: Aphididae) was used as laboratory prey, which was also collected in Fukuoka City and was reared on cucumber seedlings, *Cucumis sativus* (Family: Cucurbitaceae) in rearing cages (0.7 x 0.5 x 0.5 m). Unsexed adults of *C. septempunctata* were placed in plastic cups (12 cm diameter and 6 cm high) and were provided with aphid colonies on cucumber seedlings. Cucumber seedlings were replaced

arbitrarily to provide enough prey to the predators. When newly laid egg masses were found in cups, they were transferred to a new cup and were placed in the incubator until hatched. Neonates of *C. septempunctata* were transferred individually to Petri dishes (10 cm diameter and 2 cm high) to avoid cannibalism. They were provided water-soaked cotton balls and aphids ad libitum until they reached the developmental stages for testing.

Colonies of *Epilachna vigintioctopunctata* were reared on potato seedlings, *Solanum tuberosum*. Rearing procedures were the same as those of the aphids. Eggs were incubated for hatching and newly hatched 1st instars were used for the study. All prey and predator populations were maintained at 25±2 °C, L16:D8 photoperiod, and 65 ± 5% RH conditions inside the incubators.

All developmental stages of larvae (1st, 2nd, 3rd, and 4th instars) and adults (male and female) of *C. septempunctata* were used to assess feeding efficiency and functional responses. Feeding responses of ladybird beetles were examined by providing test ladybirds with different numbers of prey, i.e., 10, 20, 30, 40, 50, or 60 1st instars of Epilachna.

Newly eclosed adult beetles and/or newly moulted larvae of the ladybird were taken from the stock culture and placed individually in Petri dishes (10 cm diameter) for testing. They were kept in the incubator maintained at 25±2 °C and 65±5% RH, 16L: 8D photoperiod, and test individuals were starved for 24 h before testing. After 24 h, each individual in the Petri dish was provided with 10, 20, 30, 40, 50, or 60 1st instars of Epilachna collected from potato seedlings. After 24 h, ladybird beetles were removed from Petri dishes and the numbers of prey consumed were recorded. Partially or entirely eaten Epilachna larvae were counted as victims of predation. For each density treatment, 20 replicates were used, and hence, 720 ladybird beetles in all were used in the experiment.

Statistical analysis

Three types of functional response curves were fit against the number of prey consumed (N_a) and initial prey density (N_0) [34, 68, 69] to examine which type of functional response was the best fit. It was determined through a logistic regression between the proportions of prey consumed (N_a/N_0) and initial prey density (N_0) [34]. P_0 , P_1 , P_2 , and P_3 are intercept, linear, quadratic, and cubic coefficients, respectively (Eq. (1)).

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

Then, Holling's disc equation (Eq. (2)) [26] was applied to model the relationship between prey consumption (N_a) and initial prey density (N_0). Linear regression of $1/N_a$ against $1/N_0 T$ was applied. The intercept is the initial estimate of T_h/T and the reciprocal of the regression coefficient (slope) is an estimate of a . Then, the attack rate, handling time, and maximum capture rate were calculated based on nonlinear regression:

$$N_a = \frac{a N_0 T}{1 + a N_0 T} \dots \dots (2) \longrightarrow \frac{1}{N_a} = \frac{1}{a} \cdot \frac{1}{N_0 \cdot T} + \frac{T_h}{T} (y = ax + \beta)$$

where

N_a = prey consumption

N_0 = initial prey density

T = total time taken for consumption

a = attack rate

 T_h = handling time

JMP Pro 14 software was used to analyze the data and the graphs and figures were drawn with this software. The Shapiro-Wilk normality test was used to assess the normal distribution of data on the mean number of prey consumption and predation percentage by *C. septempunctata*. After assessing data were distributed as non-normal ($p < 0.05$), they were subjected to a Kruskal-Wallis test followed by a Tukey-Kramer Honestly Significant Difference (HSD) test ($p < 0.05$) for mean separation. Statistical treatments were made to examine whether significant differences could be detected in the number of prey consumption among different prey densities at each predator stage and among predator stages at each prey density. Statistical analyses were done using Statistix 8 software package.

Results

Prey consumption by *C. septempunctata* increased as prey density increased, regardless of the developmental stages of the predator, though the rate of increase showed a decreasing

trend around the highest densities tested (Fig 1). Regression lines were then fitted based on the estimates from a cubic logistic model (Eq. (1)). The proportions of prey consumed were inversely correlated with the prey densities offered, regardless of ladybird development stages. They were greater at lower prey densities but lowered at higher prey densities (Fig 2). Estimates of the linear parameter P_1 were significantly negative for all developmental stages/sexes of *C. septempunctata* (Table 1). The linear coefficient determines the type of functional response. If it is not significant, it means Type I; significantly negative means Type II; and significantly positive, and the quadratic term is significantly negative means Type III [34]. Therefore, all the stages/sexes expressed Type II functional response except 1st instar which was Type I (Table 1). The data of prey consumption by *C. septempunctata* fitted Holling's disc model (Eq. (2)) and the Type II functional response (Fig 3, Table 2). The R^2 values 0.90, 0.82, and 0.89 were higher for 4th instar, female, and male respectively (Fig 3, Table 2). The intercept and slope values of Holling's disc model were used to calculate the attack rate, handling time, and maximum capture rate.

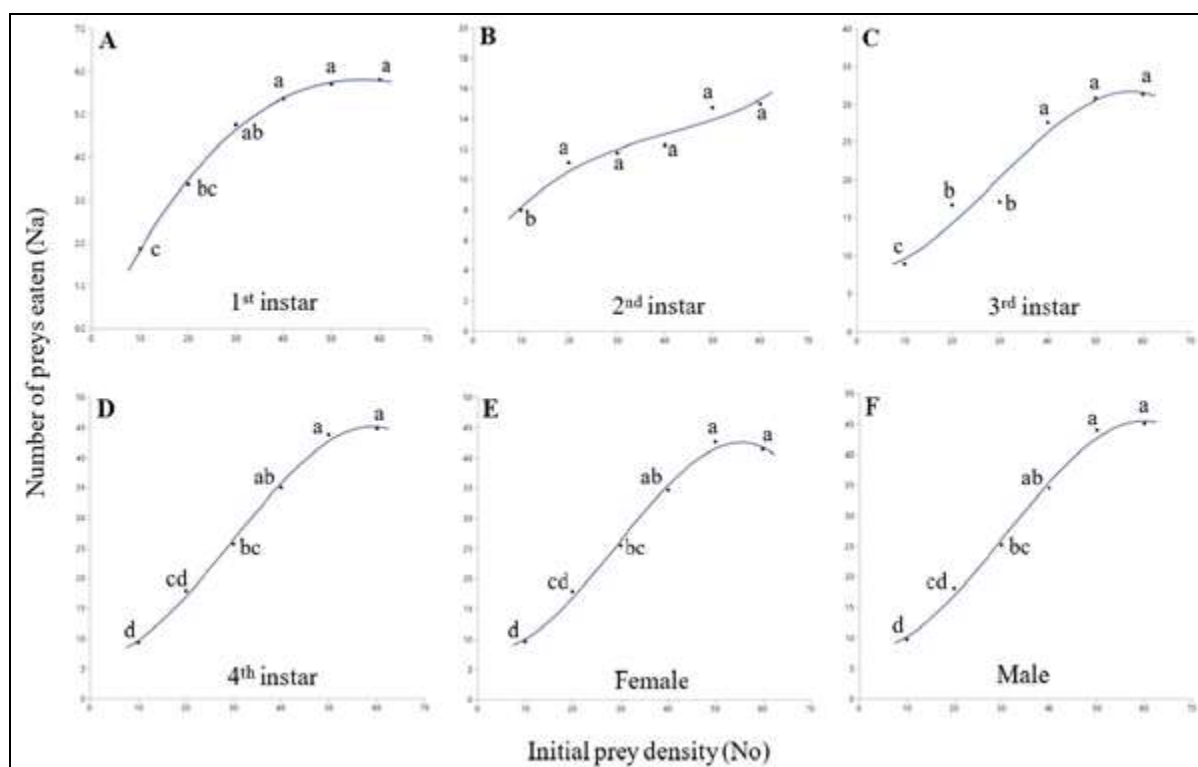


Fig 1: Mean number of prey consumption of (A) 1st instar, (B) 2nd instar, (C) 3rd instar, (D) 4th instar, (E) Female, and (F) Male of *C. septempunctata* to increasing density of 1st instar larvae of *E. vigintioctopunctata*.

The attack rate (a) of the 2nd instar of *C. septempunctata* was ranked highest (0.066) and followed by males (0.043), 3rd instar (0.043), females (0.042), 1st instar (0.040), and 4th instar (0.039) (Table 3).

The handling time (T_h), calculated by linear regression method, ranged from 0.089 h to 4.7 h (Table 3). The 4th instar of *C. septempunctata* was estimated to require the least time to handle their prey (0.089 h), and the time was followed by male (0.141 h), female (0.154 h), 3rd (0.434 h), 2nd (1.54 h), and 1st instar (4.7 h) (Table 3). The female and male adults of *C. septempunctata* showed similar time (0.154 h vs 0.141 h). It inferred that the 4th instar was most voracious whereas the 1st instar took the longest time to handle the prey, probably

due to their small mouth parts and low energy requirement (Table 3).

The theoretical maximum predation rate (T/T_h) was highest (268.8) in the 4th instar followed by males (170.4), and females (155.7). The lowest was 5.1 of *Epilachna* larvae for 1st instar per day (Table 3). The values of the coefficient (R^2) increased with the developmental stage from 0.03 to 0.97 and they were 0.93, 0.92, and 0.97 for 4th larvae, female, and male, respectively (Table 3). The high values indicated that Holling's equation adequately described the functional response of the different stages of *C. septempunctata* feeding, except 1st instar stage in which the value was 0.03.

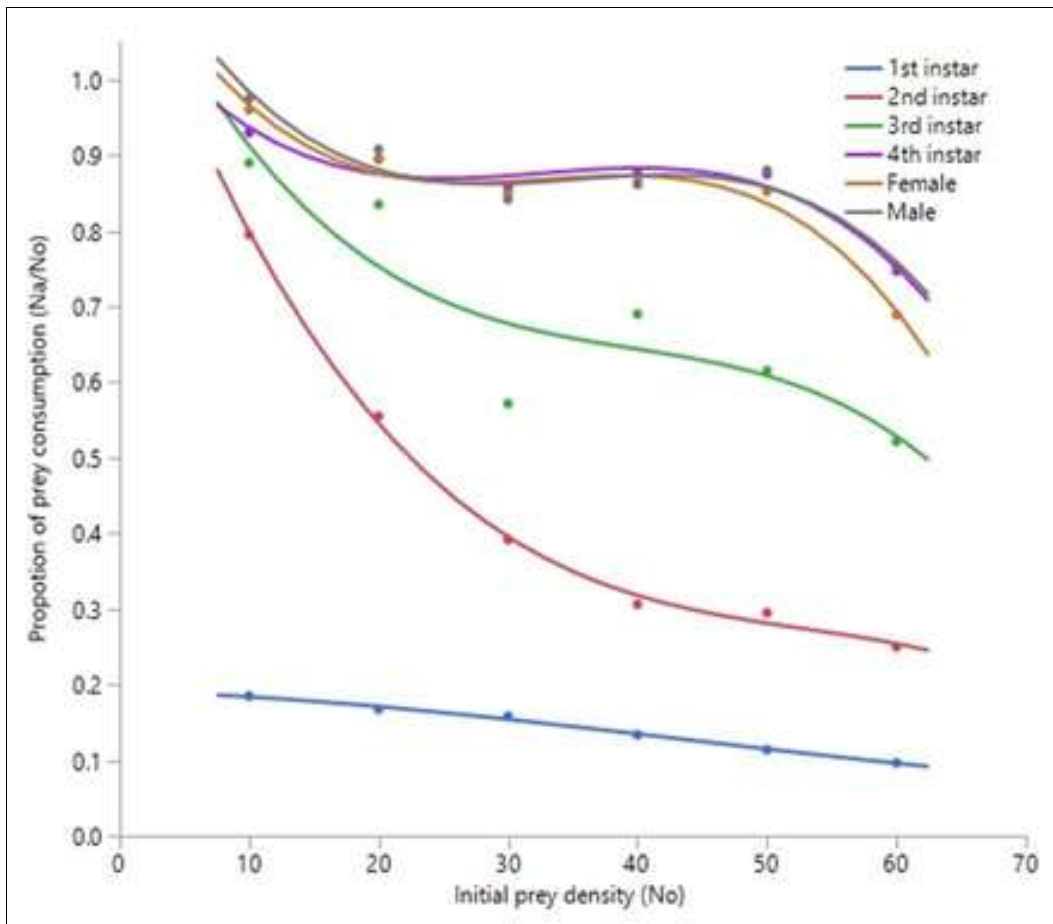


Fig 2: Mean proportions of *E. vigintioctopunctata* consumed by 1st, 2nd, 3rd, 4th instars, female, and male of *C. septempunctata* at varying *Epilachna* densities. The lines derived by the cubic logistic regression model describe Type I for 1st instar and Type II for other stages of *C. septempunctata*. See the text for the details

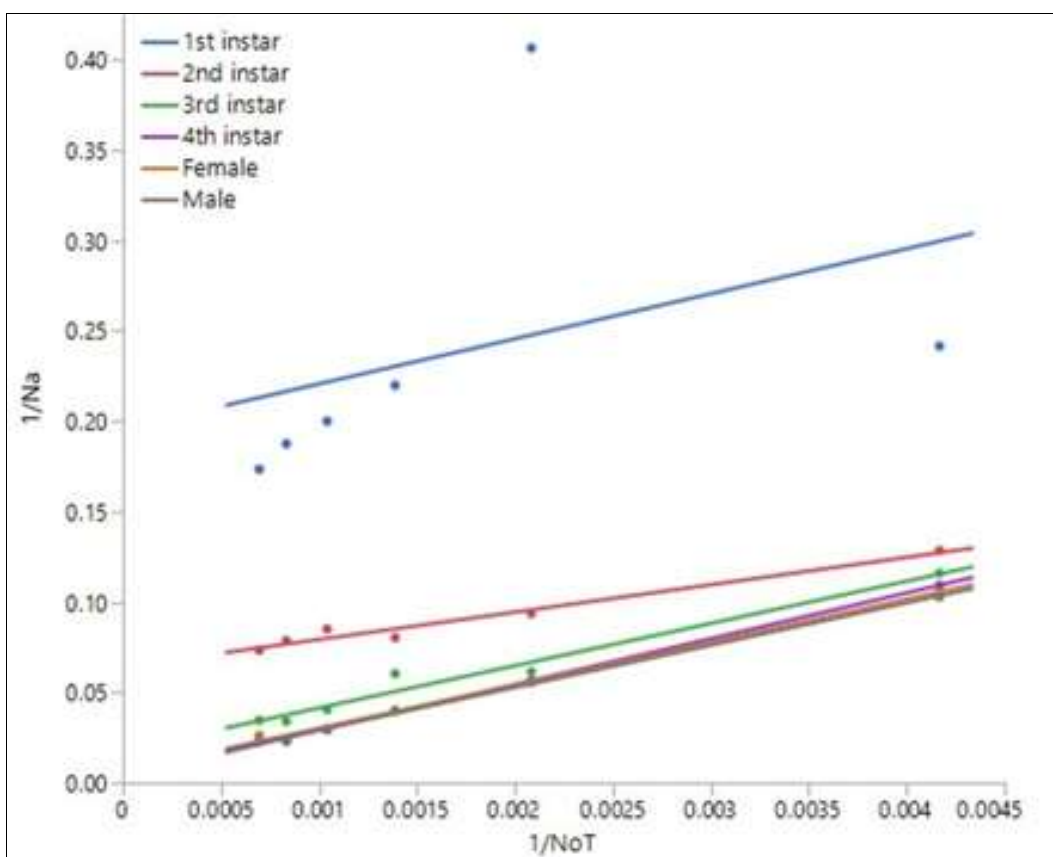


Fig 3: Reciprocal transformation of prey density (1/No) offered to 1st, 2nd, 3rd, 4th instars, female, and male of *C. septempunctata* vs consumed (1/Na) in time T.

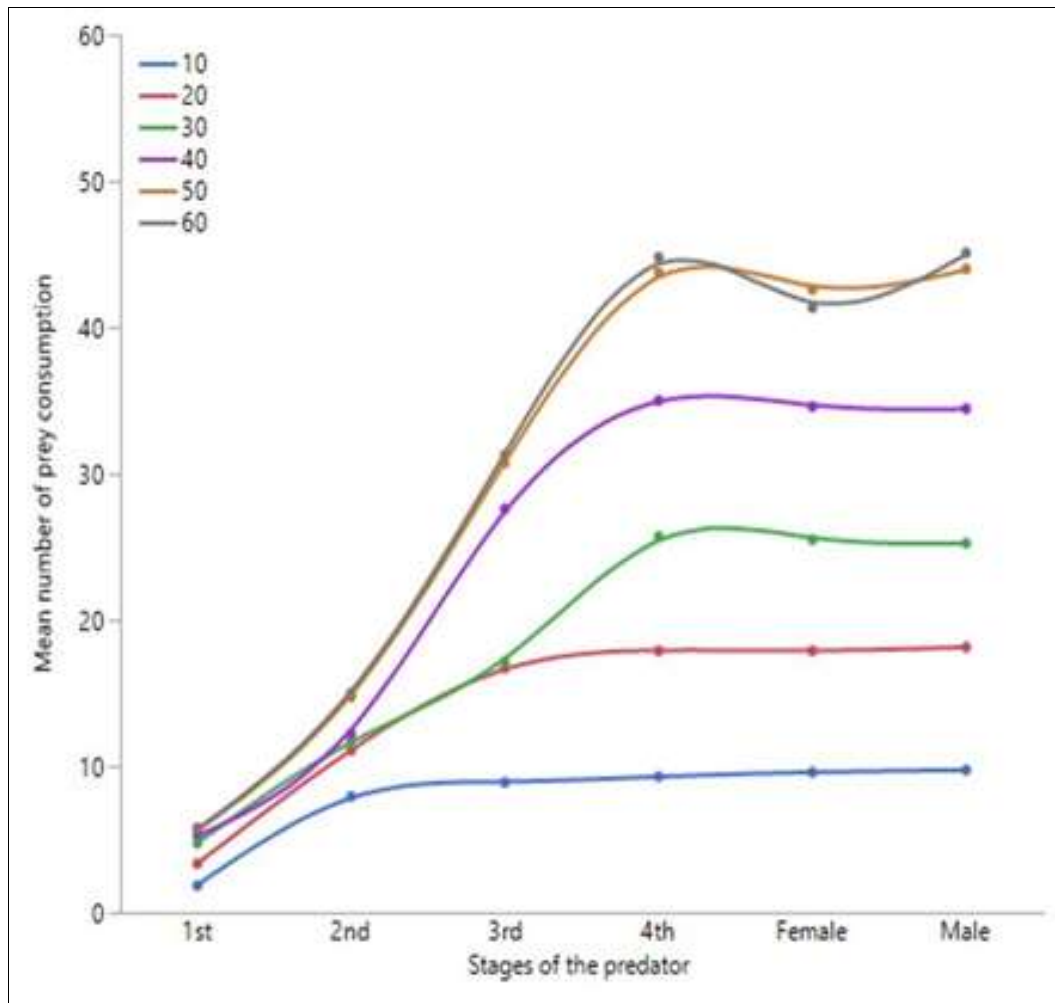


Fig 4: The mean number of prey consumed by each stage of *C. septempunctata* to initial density (10, 20, 30, 40, 50, and 60) of 1st instar larvae of *E. vigintioctopunctata*

Table 1: Parameter estimates from cubic logistic regression for the proportion of prey consumption as a function of initial prey densities by 1st, 2nd, 3rd, and 4th instar larvae and adults (female and male) of *C. septempunctata* feeding on 1st instar larvae of *E. vigintioctopunctata* to determine the type of functional response.

Stages	Parameters	Estimates	± SE	R ²	Wald Chi Square value	P value	Functional response
1 st instar	Intercept (P ₀)	0.191	0.061	0.144	9.740	0.0018	Type I
	Linear (P ₁)	-0.0004	0.007		0.003	0.9592	
	Quadratic (P ₂)	-3.771e-5	0.0002		0.029	0.8658	
	Cubic (P ₃)	2.886e-7	2.109e-6		0.019	0.8912	
2 nd instar	Intercept (P ₀)	1.187	0.081	0.785	213.098	<0.0001	Type II
	Linear (P ₁)	-0.047	0.009		25.302	<0.0001	
	Quadratic (P ₂)	0.001	0.0003		7.852	<0.0051	
	Cubic (P ₃)	-5.216e-6	2.800e-6		3.469	0.0625	
3 rd instar	Intercept (P ₀)	1.200	0.130	0.369	85.045	<0.0001	Type II
	Linear (P ₁)	-0.037	0.015		6.087	0.0136	
	Quadratic (P ₂)	0.001	0.0005		3.238	0.0719	
	Cubic (P ₃)	-7.176e-6	4.483e-6		2.562	0.1095	
4 th instar	Intercept (P ₀)	1.105	0.092	0.194	143.688	<0.0001	Type II
	Linear (P ₁)	-0.024	0.011		5.122	0.0236	
	Quadratic (P ₂)	0.001	0.0003		5.254	0.0219	
	Cubic (P ₃)	-7.87e-6	3.176e-6		6.141	0.0132	
Female	Intercept (P ₀)	1.198	0.102	0.297	138.244	<0.0001	Type II
	Linear (P ₁)	-0.032	0.012		7.764	0.0053	
	Quadratic (P ₂)	0.001	0.0004		7.503	0.0062	
	Cubic (P ₃)	-1.031e-5	3.511e-6		8.617	0.0033	
Male	Intercept (P ₀)	1.227	0.085	0.281	206.599	<0.0001	Type II
	Linear (P ₁)	-0.033	0.010		11.665	0.0006	
	Quadratic (P ₂)	0.001	0.0003		9.928	0.0016	
	Cubic (P ₃)	-9.282e-6	2.941e-6		9.959	0.0016	

Table 2: Parameter estimates from linear regression of $1/Na$ as a function of $1/NoT$ by 1st, 2nd, 3rd, and 4th instars larvae and adults (female and male) of *C. septempunctata* feeding on 1st instar larvae of *E. vigintioctopunctata* to determine the attack rate, handling time and maximum capture rate.

Stages	Parameters	Estimates	± SE	R ²	Wald Chi-Square value	P value	Lower 95%	Upper 95%
1 st instar	Intercept	0.196	0.029	0.027	46.975	<0.0001	0.140	0.252
	Slope	24.943	13.756		3.288	0.0698	-2.017	51.904
2 nd instar	Intercept	0.064	0.004	0.349	262.580	<0.0001	0.056	0.072
	Slope	15.161	1.906		63.282	<0.0001	11.425	18.896
3 rd instar	Intercept	0.018	0.002	0.783	58.792	<0.0001	0.013	0.023
	Slope	23.396	1.134		425.624	<0.0001	21.174	25.619
4 th instar	Intercept	0.004	0.001	0.932	8.035	0.0046	0.001	0.006
	Slope	25.358	0.632		1611.474	<0.0001	24.120	26.596
Female	Intercept	0.006	0.001	0.920	22.987	<0.0001	0.004	0.009
	Slope	23.826	0.645		1364.744	<0.0001	22.562	25.090
Male	Intercept	0.006	0.001	0.968	51.949	<0.0001	0.004	0.007
	Slope	23.429	0.392		3574.034	<0.0001	22.661	24.197

Table 3: Estimates of the functional response parameters, attack rate (a), handling time (T_h), and maximum capture rate (T/T_h) of different stages of *C. septempunctata* feeding on 1st larvae of *E. vigintioctopunctata* with asymptotic 95% confidence intervals using the Holling's disc model. R² is the coefficient.

Stage	R ²	Holling's disc equation	a	T _h (h)	T/T _h
1 st instar	0.03	y=0.196 + 24.94x	0.040	4.700	5.106
2 nd instar	0.35	y=0.064 + 15.16x	0.066	1.540	15.588
3 rd instar	0.78	y=0.018 + 23.4x	0.043	0.434	55.360
4 th instar	0.93	y=0.004 + 25.36x	0.039	0.089	268.839
Female	0.92	y=0.006 + 23.83x	0.042	0.154	155.676
Male	0.97	y=0.006 + 23.43x	0.043	0.141	170.425

Table 4: Percentage of prey consumption (± SE) by all predatory stages of *C. septempunctata* to *E. vigintioctopunctata* initial density

Prey density	1 st instar	2 nd instar	3 rd instar	4 th instar	Female	Male
10	18.50±3.35 a	79.50±2.66 a	89.00±3.32 a	93.00±2.42 a	96.00±2.22 a	97.50±1.23 a
20	16.75±1.79 ab	55.50±2.38 ab	83.50±2.97 ab	89.50±2.32 a	89.50±2.35 ab	90.75±2.00 ab
30	15.83±0.007 a	39.17±2.78 bc	57.17±2.36 c	85.67±2.94 ab	85.00±2.04 bc	84.17±2.13 bc
40	13.37±0.007 ab	30.63±1.44 cd	69.00±5.00 bc	87.50±3.01 a	86.50±2.81 abc	86.13±2.98 bc
50	11.40±0.006 ab	29.50±2.33 cd	61.50±2.85 c	87.50±1.93 ab	85.20±2.92 abc	88.00±2.31 bc
60	9.67±1.29 b	25.00±1.68 d	52.17±3.18 c	74.67±2.59 b	68.92±4.15 c	75.17±2.99 c

Means within the column followed by the same letter are not significantly different (Tukey's HSD Test, $p < 0.05$).

Mean numbers of prey consumed by *C. septempunctata* increased with increasing prey densities, regardless of the predator's developmental stage. Also, the predator's stage had a large effect on prey consumption. 1st instar of *C. septempunctata* attacked and consumed the smallest number of prey, ranging from 1.85 to 5.80 *Epilachna* (Fig 1).

The highest consumption was found for males (ranging from 9.75 to 45.1 prey), followed by 4th instar (9.3-44.8) and females (9.6-42.6) (Fig 1). There were no significant differences in the mean numbers among males, females, and 4th instars. In terms of the number of prey consumed during (24 h), therefore, the last instar and adult *C. septempunctata* were the most superior predators, and the maximum mean numbers they predated a day was around 45 *Epilachna* larvae (Fig 1, Fig 4). Likewise, the percentages of prey eaten depended on the developmental stage of the ladybird. The lowest percentage was observed in 1st instar (9-18%) whereas the highest prey consumption was noted in males (75-97%), 4th instar (74-93%), and females (68-96%) (Table 4).

Both female and male *C. septempunctata* showed similar patterns of predation (Fig 1, 2, 3, and 4). With the prey density increased, their predation rate increased with the decreasing rate. Both female and male *C. septempunctata* showed similar attack rates (0.042 vs 0.043) and shorter handling time (0.154 h vs 0.141 h) (Table 3) with a similar functional response curve (Fig 1E vs 1F), indicating that sexual difference was not detected in *C. septempunctata*

functional response. The percentages of prey consumed varied between 68-96% and 75-97% in females and males, respectively, depending on the number of prey, the values were similar (Table 4). Taken together, sexual differences in predation response did not exist in adult *C. septempunctata*, at least, under our experimental conditions.

Discussion

Although ladybird beetles are commonly known as predators of aphids, most aphidophagous coccinellids are not strict specialist predators [25]. They often feed on non-aphid insects. For example, *Coccinella septempunctata* preys on a variety of aphid species [6, 76] but also is found to feed on non-aphid prey [15]. The present study confirms that *C. septempunctata* also attacks and preys on *Epilachna* beetles under laboratory conditions. The result suggests that this predator has the potential as an important natural enemy of *Epilachna* beetles. One of the main purposes of our study is to determine the type of functional response of *C. septempunctata* when it encounters *Epilachna* larvae. The present study gives evidence that *C. septempunctata* mostly shows Type II response. The potential of a biocontrol agent is determined based on several ways, including functional response tests [24, 13].

Functional response test suggests whether a given natural enemy may be useful as a biocontrol agent at low densities of pests [57]. Generally, useful natural enemies should

demonstrate a Type II functional response that may be linked to successful control of prey populations [27]. Type II functional response indicates the initial increase in the rate of prey consumption at lower prey densities, which is one of the key features of a successful agent of control since suppression of a target pest at the initial or early stage of population increase is important in the overall control of the pest [51, 58]. Therefore, the present data suggest that *C. septempunctata* can be useful in controlling Epilachna.

Previous studies with *C. septempunctata* have demonstrated that the larvae and adults exhibit Type II functional responses to aphids [6, 20, 21]. Our study also shows that this is the case for non-main prey item, Epilachna. Also, Type II functional response was reported in other ladybird species [28, 56]. In addition, a previous study proved that *C. septempunctata* did not show any stage-specific response [52]. However, in our study, 1st instar of *C. septempunctata* expressed Type I functional response whereas all other development stages exhibited Type II functional response against *E. vigintioctopunctata*. A similar result was observed for the harlequin ladybird *Harmonia axyridis*; the functional response by the first instar was Type I whereas the other larval stage and adult showed Type II on the aphid *Myzus persicae* [65]. Because multiple factors influence the functional responses of predators [70], further studies should be needed to reveal what factors are involved in the stage-dependent response.

Attack rate, handling time, and maximum predation rate are the key parameters of functional response. Natural enemies with a high attack rate (a) and low handling time (T_h) can be the most effective biocontrol agents [5, 66]. In the present study, we showed two important parameters, *i.e.*, a and T_h , varied with predators' developmental stage (Table 3). The a was higher in the 2nd instar and was lower in the 4th instar. Similarly, developmental stage dependency has also been reported for several ladybird beetles. However, various responses have been recorded for coccinellid beetles, which may lead to confusion. For example, lower attack rate and greater handling time were observed for 4th instars than adults in *Coleomegilla maculata* DeGeer [45] whereas the reverse result was true in *Scymnus creperus* Mulsant [74]. Fourth instars exhibited a higher search rate and greater handling time than adults of *H. axyridis* [39] and *Hippodamia convergens* [73]. Hence, our study suggests the importance of the developmental stage in considering the efficacy of natural enemies in the context of functional response.

In many cases, it is generally shown that predators with a short handling time may have a high attack rate and vice versa [6]. Likewise, in *C. septempunctata*, the 4th instar [21] and female [20] have the highest attack rate with the lowest handling time among the developmental stages examined. In our study, however, we found a low attack rate with a low handling time in the 4th instar of *C. septempunctata*. A major difference between the previous and our study is the prey type used. In our study, we used Epilachna as an alternative prey. We suggest that the discrepancy should arise due to a difference in prey type, *i.e.*, common (probably preferred) and aphid prey versus alternative, rather tentative prey items. Thus, our study highlights the importance of prey type on the functional response of natural enemies.

The nutrient requirement should depend on the development stage of the predator. Younger stages of arthropod predators like ladybird beetles are smaller in size and show slower movement, longer prey handling time, lower nutritional requirements, and lower digestive potential [25, 39, 51]. These

would be the reason for the smallest mean prey consumption and lowest consumption rates of 1st instar of *C. septempunctata* demonstrated in the present study (Fig 1). However, the attack rate of 1st instar was more or less equal to the later and adult stages of *C. septempunctata*. Therefore, the attack rate was not a good indicator of the predator efficiency of *C. septempunctata* in this study.

A possible reason is the involvement of the hunger level of predators. In our experimental design, test predators in Petri dishes were not provided with prey for 24 h before testing, and this treatment should make their hunger level and energy requirement high, leading to a quick predation response and thereby a high predation rate of *C. septempunctata*. In addition, hunger levels may vary according to the predator stage or size, which should affect the attack rate. Our study therefore suggests hunger level of predators in a short-term experiment (in our study, 24 h observation) may have a significant impact in estimating the attack rate.

Handling time was shorter for later stages of development (Table 3). The chance of encountering prey may increase with the body size of the predator because it should positively affect the speed of movement and the recognition of target prey via eyesight or smell [4, 10, 13, 19, 62]. Also, handling time should decrease with the increasing body size because a larger size allows the predator to handle the prey more easily and subdue it more quickly [22, 62]. Thus, 4th instars and adult *C. septempunctata* are superior in terms of prey handling.

In addition, the 4th instar of *C. septempunctata* was more voracious than the earlier larval stages and responsible for a 60-80% predation rate [25]. This should be due to their extra requirement of nutrients during their development and subsequent non-feeding pupal stage [25]. A similar trend was observed in our study. After 24 h, the 4th instar consumed 74-93% of Epilachna larvae. Also, consumption rates for adult males and females were 68-96%, and 75-98%, respectively (Table 4). 4th instar and adults of *C. septempunctata* should be major consumers, with a similar efficiency of consumption due to high nutritional requirements for their growth and reproduction [44, 53]. Since these stages can move faster than the other stages, their capability of controlling larval Epilachna should be higher even when Epilachna occurs at low density.

Although, in the present study, the prey consumption of *C. septempunctata* increased with Epilachna density, regardless of their development stage, the rates of increase showed a decreasing trend (Fig 1). This may be explained by the change in handling time with the number of Epilachna larvae consumed during a 24 h experimental period. Hungry predators devour the first prey they encounter, and the initial handling time should be short. As they consume more prey, the time of handling can be longer, and finally, due to satiation, the prey density-handling time curve reaches an asymptote [25, 51, 57]. Our study demonstrated that 4th instar and adult *C. septempunctata* reached a level of satiation at a prey density of 50 (Fig 1). This value is lower than the values reported in previous studies, in which preferred aphid prey was used [9, 36].

The maximum consumption rate of insect predators is inactive after reaching satiation or their consumption threshold [75] since they are digestion-limited [55]. Hence, digestion ability could affect their foraging efficiency [16, 33]. The increasing digestion rate could decrease the prey handling time and thus increase the rate of prey encounters [47]. The 4th larval instar and adults of *C. septempunctata* were faster in subduing and

digesting *Epilachna* due to their development in the digestion system with their age. The same result was recorded in different stages of *Saduria entomon* (L.) especially at high prey densities^[4, 33] found that subduing and digesting prey did not directly impede the predator from further searching for or handling prey due to partial consumption resulting in a greater reduction in pest abundance^[4, 25].

Prey activity has a significant impact on the vulnerability of ladybird beetles^[59]. In general, sclerotized cuticles of prey insects can function as protection against predation^[18]. However, the first instars of *E. vigintioctopunctata* just after hatching should be much less sclerotized, rather soft, and easy to handle prey for *C. septempunctata*. Instead, they are very small items with low biomass, and, therefore, the predators must consume many prey individuals to obtain enough amount of nutrients for their satiation. Then, functional response to *Epilachna* may differ depending on the developmental stage of *Epilachna*, and this is a future subject. Most of the studies proved that females are more voracious than other stages^[39]. However, in our study, there was no significant difference in voracity between females and males. But 4th instar of *C. septempunctata* handled prey in less time than adult females and males. Hence, the 4th instar is a more competitive stage than other stages and has a greater killing power.

Finally, the present study inferred that the 4th instar and adult stages of *C. septempunctata* are the most important and voracious stages for the management of *Epilachna*. Also, the relative rate of prey consumption by *C. septempunctata* was higher at lower densities, which indicates that this predator would be more effective at controlling the *Epilachna* population at lower densities. Therefore, to obtain effective biocontrol of *Epilachna* with the coccinellids, they should be released when *Epilachna* density is relatively low if an inoculative biocontrol program is applied. Alternatively, the conservation of *C. septempunctata*, which is commonly found in most arable lands, should be done with, for example, an integrated pest management program. Although functional response is an important tool for evaluating natural enemies, laboratory studies cannot be directly extrapolated to natural field conditions because they may not accurately reflect field predation rates. Therefore, field investigations are needed to evaluate how *C. septempunctata* can contribute *Epilachna* management.

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Author's contribution

JCP: Conceptualization, Experimental design, Data collection and analysis, Funding acquisition, Writing-original draft. TU: Conceptualization, Experimental design, review, and editing. All authors read and approved the final manuscript.

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