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Life history fitness of giant ladybird predator (Coleoptera: Coccinellidae) of woolly aphids (Hemiptera: Aphididae) in varying prey densities from northeast India

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Quantity of natural prey available to insect predators varies in time and space and it influences the fitness of individual predators. This was examined for *Anisolemnia dilatata* (Fab.) which is a specialist predator of woolly aphids of bamboo plants and sugarcanes, and endemic to south Asia and Asia-Pacific regions. Results of a laboratory study using 45 larvae and 10 adult females showed that individual *A. dilatata* larvae performed best at an optimal density of 250 live aphids per larva per day, and adult females from these larvae matured faster and produced higher number of viable eggs than the larvae that grew and

developed at sub-optimal prey density of 200 or less aphids. About 73–80% of the larvae that survived at the density of 50 or 100 or 150 aphids per day took significantly longer time to complete development and to reach the age at maturity. Such females were significantly smaller in size and produced fewer viable eggs. Results showed that larvae and adults of *A. dilatata* required high density of aphid prey to support its optimal life history fitness. Results hold promise in the application of this predator in the control of waxy aphid pest of cultivated bamboos and sugarcanes.

Keywords: Bamboo plants, giant ladybird predator, life history fitness, prey requirement, woolly aphids prey.

ANISOLEMNIA dilatata (Fab.) (Coleoptera: Coccinellidae) is one of the two prevalent giant ladybird beetle predators of waxy aphids that occurs in tropical seasonal forests of South, South East and Far East Asia^{1–3} which contain bamboos as one of the principal component of vegetation complex. The other species is *Synonycha grandis* Thunberg⁴. Giant ladybird beetles are unique among ladybird predators due to their large size and prey specialization of woolly aphids infesting bamboos and sugarcanes⁵. Imagos of *A. dilatata* measure about 3.5 times (mean \pm SE = 137.43 \pm 0.68 mg, n = 45) in comparison to the average size of aphidophagous *Coccinella septempunctata* L. (mean \pm SE = 39.42 \pm 1.22 mg, n = 20) that feeds on several aphid species in diverse habitats^{6,7}. Out of 78 species of bamboos under 19 genera recorded from northeast India, *Bambusa balcooa* Roxb., *B. aurandincea* (Retz.) Willd. and *B. tulda* Roxb. are widely cultivated and used for economic purposes in Tripura^{8–10}, a province in the south of northeast India. Wool producing horned aphid species, *Ceratovacuna silvestrii* (Takahashi), *C. indica* (Ghosh, Pal & Raychaudhury), *Paraoregma alexendari* (Takahashi) and *Pseudoregma bucktoni* (Takahashi), make dense colonies on young leaves and tender shoots of perennial bamboo species. Among the several natural enemies of woolly aphids that have been recorded on bamboo plants, larvae and adults of *A. dilatata* are found to be the dominant predators (Figure 1 a and c)¹¹.

Biology and ecology of smaller species of ladybird predators of aphids and coccids are well known with respect to their functional and numerical responses to different prey species^{12,13}, but no information exist on the life history fitness parameters of the giant ladybird species, *A. dilatata*. In a field study from Tripura, Majumder and Agarwala¹¹ showed that the incidence of *A. dilatata* on two bamboo species, *B. tulda* and *B. balcooa*, was restricted to high density phase of aphid population of *C. silvestrii*; thus populations of predator and prey co-existed for only 27 weeks out of 44 weeks incidence of the aphid prey in a year of study. Due to economic importance of bamboo plants as means of livelihood of large number of rural folk in parts of south Asia⁸ and great prey-feeding potential of *A. dilatata*^{4,5}, a laboratory study

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was conducted to evaluate its life history fitness, viz. aphids eaten by fresh weight by larvae and adult females, development time, larval growth rate, adult female size by fresh weight at pupa eclosion and at maturity, total fecundity, egg size, and hatching success of eggs at different prey densities that are likely to occur in the field.

Ten pairs of males and females of *A. dilatata* collected from the forest at Ishanchandranagar (23°45.669'N and 91°15.967'E, Ele: 34 m) near the Tripura University Campus were used to establish stock culture in an environmental chamber maintained at 22°C ± 1°C temperature, 65 ± 2% RH and 16 : 8 h L : D photoperiod. Beetles were kept in a ventilated plastic container (25 cm length × 15 cm width × 12 cm height) and these were offered *ad libitum* live *C. silvestrii* aphids that were collected from infested bamboo plants in the field. Beetles readily ate woolly aphids and females laid eggs in batches on corrugated papers¹⁷ kept in the container. Eggs were transferred to 9 cm diameter paired petri dishes (one batch per dish). On hatching, 30 larvae were kept singly in 9 cm diameter petri dishes lined on bottom with slightly moistened filter paper and reared to pupation on a surplus supply of woolly aphids placed on fresh bamboo leaves. Aphid prey was replaced at 24 h interval when petri dishes were cleaned. Developing larvae were observed for any mortality twice a day, at 9 am and, again, at 5 pm till pupation. Pupae were kept undisturbed for the emergence of adults. Ten females of similar sizes and an equal number of similar aged males from these adults were kept in pairs in ventilated culture dishes (15 cm diameter × 1.5 cm height), one pair per petri dishes and were maintained for two generations in order to acclimatize the beetles to the laboratory conditions. Eggs obtained from the second generation females were used in the experiments.

Forty five recently hatched larvae (<12-h old) were kept singly in 5 cm diameter paired petri dishes. These were reared till pupation at each of the six initial prey

densities of 50, 100, 150, 200, 250 or 300 live aphids placed on fresh bamboo leaves to correspond to the variations in prey population on bamboo plants recorded in the field¹¹. Lower prey quantity was not used in the experiment because a preliminary laboratory study revealed 80% mortality in development at a supply of 40 aphids per larva per day and a few adults that survived did not reproduce ($n = 20$). Larvae of different instars and adult females were provided aphids to match their prey capture efficiency¹²; thus, first and second instars were offered a random mix of smaller aphid preys of second and third instars whereas third and fourth instars and adult beetles were offered a random mix of fourth instar and adult aphid prey. Different instars and adults of aphid prey were distinguished and counted under a tabletop magnifying glass based on differences in body length, wax gland structure and length of frontal horns in head⁴. In 6 treatments, a total of 270 larvae were used. Numbers of aphids eaten by fresh weight by individual larvae in development and by adult females in their life time at different prey densities were recorded at 24 hours interval.

Ten females of approximately similar size by fresh weight were used in each of the six treatments. These were maintained singly in 10 cm diameter petri dishes on respective initial prey density till death. Thus, 60 females were used in 6 treatments. Females were paired with similar aged males taken from the stock culture and kept for 2 h at the time of food change to allow mating and to maintain reproductive vigour in experiments. Weights of larvae (<12 h old) used at the start and in the pre-pupa stage, and of adults soon after eclosion (<12 h old) and at death were recorded in a Mettler Toledo microbalance sensitive to 2 µg. Quantity of aphids eaten every 24 h in mg by individual larvae and adults, development time from eggs to pupa eclosion (DT) in days, larval growth rate (LGT) representing difference in weights between larvae at birth and pre-pupae stages as a ratio of development time, larval mortality in development (MORT), maturity period (MP), reproductive period (RD) and adult longevity (AL) in days, lifetime fecundity representing number of eggs produced by a female in its life time (FEC), fresh egg weight (EW) and hatching success of eggs (%) (HSE) were recorded for each adult female in the six treatments. Observations were made at 6 h intervals between 8 am and 8 pm, and any eggs deposited in treatments were removed to prevent egg cannibalism. Adults of *A. dilatata* from the experiments are preserved in the collection of the Insect Biosystematics Laboratory, Tripura University.

Parametric data, functions of which are dependent on definite parameters, met the criteria of normality and equal variance. These were subjected to one-way analysis of variation (ANOVA) to determine the effects of prey density on the life history fitness parameters, and mean values were compared using Tukey's multiple comparison tests. Non-parametric data, functions of which are not



Figure 1. *Anisolemnia dilatata*: (a) adult female, (b) a batch of eggs, and (c) a third instar larva in a high density colony of *Ceratovacuna silvestrii*.

Table 1. Mean (\pm Sem) values of life history parameters of larvae and adults of *A. dilalata* reared on different density of woolly aphid prey, *C. silvestrii*. Values in brackets denote number of observations and different alphabets with mean values in each row as superscripts denote significant differences between the means by Tukey's multiple comparison tests. Abbreviations denoted under the

Fitness parameters	Prey density [n]							ANOVA	
	50	100	150	200	250	300	df	F ratio	P [#]
Larva									
DT (days)	21.13 \pm 0.40 ^a [33]	19.00 \pm 0.25 ^b [36]	17.30 \pm 0.17 ^c [42]	15.62 \pm 0.18 ^d [45]	14.84 \pm 0.16 ^d [45]	15.24 \pm 0.16 ^d [45]	5, 240	353.22	*
LGT (mg/day)	4.36 \pm 0.06 ^a [33]	6.23 \pm 0.09 ^b [36]	8.23 \pm 0.14 ^c [42]	10.40 \pm 0.17 ^d [45]	11.28 \pm 0.08 ^e [45]	10.86 \pm 0.19 ^d [45]	5, 240	1488.11	**
Prey eaten (mg)	271.74 \pm 1.74 ^a [33]	409.66 \pm 1.32 ^b [36]	528.24 \pm 1.35 ^c [42]	580.87 \pm 0.94 ^d [45]	634.33 \pm 1.30 ^e [45]	631.24 \pm 1.29 ^e [45]	5, 240	11308.04	***
Adult female									
AW-PE (mg)	77.14 \pm 0.93 ^a [10]	99.68 \pm 0.74 ^b [10]	122.26 \pm 0.63 ^c [10]	135.09 \pm 1.72 ^d [10]	139.33 \pm 0.83 ^e [10]	137.45 \pm 0.85 ^e [10]	5, 54	2402.77	***
AW-MAT (mg)	141.20 \pm 1.10 ^a [10]	190.36 \pm 1.40 ^b [10]	208.76 \pm 1.41 ^c [10]	190.28 \pm 1.63 ^d [10]	197.24 \pm 1.99 ^e [10]	194.96 \pm 2.31 ^e [10]	5, 54	194.14	*
MP (days)	63.50 \pm 0.92 ^a [10]	45.60 \pm 1.20 ^b [10]	34.90 \pm 0.94 ^c [10]	19.80 \pm 1.03 ^d [10]	13.90 \pm 0.66 ^e [10]	15.10 \pm 1.06 ^e [10]	5, 240	403.56	**
RP (days)	39.40 \pm 1.37 ^a [10]	58.10 \pm 1.99 ^b [10]	70.70 \pm 1.55 ^c [10]	87.60 \pm 1.81 ^d [10]	93.70 \pm 1.27 ^e [10]	92.50 \pm 1.17 ^{cd} [10]	5, 240	196.99	*
Prey eaten (mg)	2938.52 \pm 37.77 ^a [10]	6093.12 \pm 30.12 ^b [10]	9431.37 \pm 26.43 ^c [10]	13060.20 \pm 69.03 ^d [10]	15238.18 \pm 62.02 ^e [10]	15131.47 \pm 73.77 ^e [10]	5, 54	8990.30	***
FEC (no.)	273.40 \pm 2.32 ^a [10]	554.80 \pm 3.39 ^b [10]	775.20 \pm 3.44 ^c [10]	981.20 \pm 3.15 ^d [10]	1177.30 \pm 4.57 ^e [10]	1165.20 \pm 3.68 ^e [10]	5, 54	10568.78	***
EW (mg)	0.692 \pm 0.003 ^a [20]	0.737 \pm 0.004 ^b [20]	0.778 \pm 0.003 ^c [20]	0.852 \pm 0.003 ^d [20]	0.881 \pm 0.002 ^e [20]	0.879 \pm 0.003 ^d [20]	5, 114	472.74	*

[#]P values are denoted by asterick (*) marks: *significance at <0.05; **significance at <0.01; ***significance at <0.001 levels.

dependent on any definite parameter, were arcsine transformed to reduce unequal variance and to ensure normal distribution. Percentage of larval mortality during larval development was analysed by a two-tailed Fisher's exact test between treatments (prey density) and control (larvae kept on surplus aphid prey), and that of HSE were analysed by post hoc Kruskal-Wallis test. A significance level of 0.05 was used to reject the null hypothesis. Data analyses were performed using Origin 7 software (Bigtech Solution, Bengaluru, India).

Means of different observations and their standard errors (Sem) values are presented in Table 1. Mean DT was the longest and mean LGT the slowest at a density of 50 aphids. DT decreased at higher prey density and the shortest DT was recorded at a density of 250 aphids per larva. In comparison, LGT increased from low to high prey densities and the fastest growth occurred at the supply of 250 aphids per larva. High mortality was recorded at lower prey density with the highest recorded at 50 aphids per larva (Fisher's exact test: 50 aphids: $P < 0.001$ ($n = 33$); 100 aphids: $P < 0.001$ ($n = 36$); 150 aphids: $P > 0.05$ ($n = 42$); 200, 250 aphids and 300 aphids: $P > 0.05$) ($n = 45$). No mortality was recorded in the first instar stage in the 6 treatments and for other larval instars at prey densities of 200, 250 and 300 aphids respectively. Mean quantity of aphid prey eaten by active larvae increased significantly from low to high prey densities in the six treatments. Maximum quantity of prey eaten by a larva was recorded at 250 aphids and this did not differ from the quantity eaten at 300 aphids per larva.

Mean sizes of adult females at pupa eclosion (AW-PE) and at maturity (AW-MAT) showed significant differences in the six treatments. The smallest sizes were recorded at a supply of 50 aphids and the largest were recorded at a supply of 250 aphids. Mean MP decreased from 62.50 days recorded at 50 aphids per female to the 13.90 days at 250 aphids per female. In comparison, mean RP increased significantly from lower to higher prey densities. Mean quantity of aphid prey eaten and lifetime fecundity (FEC) by females differed at different prey densities and these increased gradually from low to higher prey densities, with minimum and maximum values recorded at 50 aphids and at 250 aphids respectively.

Mean size of individual eggs by fresh weight (EW) and their hatching success (HSE) showed significant differences at different prey densities (ANOVA: EW: $F = 472.74$, $df = 5, 114$, $P < 0.05$; HSE: Kruskal-Wallis: $H = 56.15$, $P < 0.001$). At low prey density (50 aphids per day) females produced small sized eggs (mean \pm SE = 0.692 ± 0.003 mg, $n = 20$) with reduced viability (HSE: 53.92%). Maximum EW (mean \pm SE = 0.881 ± 0.002 mg, $n = 20$) and maximum HSE (74.20%) were recorded at the density of 250 aphids and these did not differ from the mean EW (0.879 ± 0.003 mg, $n = 20$) and HSE (72.90%) recorded at higher prey density (300 aphids per day).

The laboratory study using 45 larvae and 10 adult females as initial replicates revealed the high costs of foraging for giant ladybirds in low prey density as was evident from high mortality in development, smaller size of surviving adults, lower fecundity, smaller eggs and low hatching success rate of eggs. Number of aphids available in a patch as a food to predators varies in time and space¹³⁻¹⁷. Predators able to achieve greater fitness in a variable prey food resource will have higher sustenance and biocontrol efficiency than the others¹⁸⁻²⁰. According to Majumder and Agarwala¹¹, *A. dilatata* females required a minimum average density of 16.11 ± 0.81 aphids per leaf of bamboo plants to become gravid and lay the first eggs in field and produced maximum eggs at the density of 82.33 ± 3.61 aphids per leaf (Figure 1 b). That result fits well with the results of this study according to which life history fitness of *A. dilatata* was significantly affected at lower prey density of 50, 100 or 150 aphids per day in comparison to optimal performances recorded at a higher prey density of 200 or more aphids. Such a difference in performance may be attributed to the differences in actual amount of food available as nutrients and energy requirements of growth, development and production of eggs after meeting metabolic cost¹⁹. This explains why giant ladybird predators are prey specialists of perennial bamboo habitats in the wild that supported high aphid prey population for longer duration, up to 49 weeks in a year^{11,20}. Larvae and adults of giant ladybeetles ate aphids without being affected by their woolly secretions which deter many small size insect predators^{12,21}. Results of this study can be useful in the development of methods for mass rearing of *A. dilatata* which is a strong predator of sugarcane aphids⁵.

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Variations in the abundance and diversity of insects in apple orchards of Kumaun, Western Himalaya, India

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Availability of pollinators in a landscape plays a significant role in pollination success, which is essentially important for crops like apple with high dependence on animal-mediated pollination. Realizing this, to estimate the availability (diversity and density) of insect visitors, including pollinators, the present study included pan trap experiments and transect walks for assessment of foraging resources across eight apple orchards in Kumaun, Western Himalaya, India. It was observed that insects were attracted more towards yellow traps, and availability was highest in summer season. Apple mass flowering during summer, in spite of lower diversity of other foraging resource, helps in maintaining availability of insect groups.

Keywords: Apple orchards, insect diversity, landscape, pollinator abundance.

EFFECTIVE animal pollination is an extremely important ecosystem service (ES)^{1,2}, and is often considered endangered ES¹. Besides being important for many crops^{3,4}, it also helps in conservation of global biodiversity^{5,6}. However, rapid decline of pollinator populations has emerged as an important concern for conservation biologists^{2,7}. In this context, changing land-use patterns, human disturbances (e.g. pesticide use, habitat destruction, and resource destruction), etc. are reported as major causative factors⁸.

The availability (diversity and abundance) of pollinators in a landscape plays a significant role in pollination success^{9,10}, and landscape attributes that have an impact on the availability of pollinators become responsible for effectiveness of pollination. Lonsdorf *et al.*¹¹ have provided quantitative evidence of relationships of specific pollinator nesting resources, floral resources and foraging distances to estimate the relative abundance of pollinators and the pollination services, across agricultural landscapes. Reports suggest that close to and in natural habitats, the wild bee populations thrive well^{12,13}. The abundance of choice flowers, where colour defines the intensity of attraction for bees, also influences availability of pollinators¹⁴. Therefore, abundance of choice flowers within unit area around the study field is often used for

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