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Conspecific egg quality and distribution pattern do not affect life history traits of ladybird, *Menochilus sexmaculatus*

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Abstract

Egg cannibalism is very common among predaceous ladybirds. Studies have been done on coccinellids using egg as a diet but information on the influence of egg freshness and egg pattern on life history attributes of aphidophagous ladybird, *Menochilus sexmaculatus* (F.) (Coleoptera Coccinellidae) under laboratory conditions are scarce. In our experiment, the first instars were reared on fresh or frozen conspecific eggs in both clustered and scattered patterns. Incidence of cannibalism was not affected by either egg freshness or clustering pattern. However, percent consumption by instars differed significantly with first and fourth instar consuming more of frozen clustered and fresh scattered conspecific eggs, respectively. F2 generation offspring had similar development on frozen and fresh conspecific eggs. Females reared on frozen eggs had higher fecundity and percent egg viability whereas longevity followed the reverse trend. However, overall fitness was similar on both diets, indicating that egg freshness did not affect nutritional status.

Key words: *Menochilus sexmaculatus*, cannibalism, egg pattern, egg freshness.

Introduction

Cannibalism is a widespread phenomenon and is known across taxa from lower eukaryotes to higher primates (Fox, 1975; Dong and Polis, 1992). It is a form of competition that involves killing and gaining energy from conspecifics (Claessen *et al.*, 2004). Numerous costs of cannibalism, like loss of inclusive fitness (Joseph *et al.*, 1999; Schausberger and Croft, 2001), risk of injury and disease transmission during the attack (Pfennig *et al.*, 1998; Rudolf and Antonovics, 2007) are recognised, but there are also evolutionary benefits. These are usually in the form of competitive and nutritional advantages to cannibals, in terms of, minimising competition (Pfennig, 1997; Trumbo and Valletta, 2007), providing population stability and persistence (Ayasse and Paxton, 2002; Kang *et al.*, 2015).

Various laboratory studies and model analysis have also demonstrated benefits of cannibalism in terms of improved growth rate, survivorship, fecundity and longevity (Abdel-Salam and Abdel-Baky, 2001; Wu *et al.*, 2013). Adults of red flour beetle, *Tribolium castaneum* (Herbst) are known to be more fecund when allowed to eat eggs as larvae (Ho and Dawson, 1966). Predation of conspecific eggs in apple snail is a usual behaviour (Cazzaniga, 1990). Even the larval stages of *Drosophila melanogaster* Meigen have been reported to attack conspecific eggs under starved conditions (Ahmad *et al.*, 2015). Immature stages of insects are generally considered to be more vulnerable to cannibalism as compared to the adult because of reduced mobility (Dixon, 2000; Dixon and Agarwala, 2002), poor foraging capacity and degree of spatial co-occurrence between stages (Dixon, 2000; Osawa, 1993).

In ladybird beetles, cannibalism of egg, larval and pupal stages are frequent in the laboratory as well as in fields (Dixon, 2000), with egg cannibalism being quite common (Michaud and Grant, 2004; Omkar *et al.*, 2006; Santi and Maini, 2007). Cannibalism in ladybirds has been demonstrated to be a function of relative vulnerability and frequency of encounters (Agarwala and Dixon, 1993). Studies on different components of cannibalism in ladybirds have revealed that it is largely dependent on the species, food availability (Agarwala and Dixon, 1992; Devee *et al.*, 2018), degree of relatedness (Pervez *et al.*, 2013), size disparity and larval density (Michaud, 2003; Pervez *et al.*, 2006). Even in the presence of a sufficient amount of essential prey, ladybirds are known to preferably consume conspecific eggs (Santi *et al.*, 2003; Omkar *et al.*, 2006). This has been attributed to their high nutritional suitability (Omkar *et al.*, 2006; Ahmad *et al.*, 2015) as first meal to early instars by increasing their chances of survival (Agarwala and Dixon, 1992; Omkar *et al.*, 2006). It has also been reported that the egg laying pattern also influences predation in ladybirds, with clusters being less attractive to heterospecific predators (Agarwala and Dixon, 1993). It is well recognised that quality as well as quantity of diet influences the life history traits of ladybirds (Dixon, 2000; Omkar and Bind, 2004; Hamasaki and Matsui, 2006; Castro-Guedes *et al.*, 2016).

In view of ladybirds predilection to cannibalism of eggs, the nutritional status of eggs and the well-established effect of food quality on development, we decided to assess the effect of conspecific egg diet on immature as well as adult stage of ladybird, *Menochilus sexmaculatus* (F.) (Coleoptera Coccinellidae). This species was selected as an experimental model due to their abundance in local fields, high reproductive output, and wide prey range (Omkar and Bind, 2004; Omkar *et al.*, 2005), also incidences of cannibalism were observed among immature stages in fields as well as laboratory. We also presented eggs either as frozen or fresh and in scattered or clustered pattern to assess ladybird preference for prey. It has earlier been observed that ladybirds cannot detect frozen aphids (Stubbs, 1980).While suitability of an egg versus conspecific and heterospecific diet has been assessed previously in ladybirds (Michaud, 2003; Felix and Soares, 2004; Omkar *et al.*, 2004) there is, to the best of our knowledge, none on frozen versus fresh eggs. It is our hypothesis that scattered eggs would be preferred more as a prey as they would not give out aposematic signals as suggested by Agarwala and Dixon (1993). Also, fresh eggs would be more preferred and suitable as food, since freezing may deteriorate egg nutritional quality. Contrary reports are available on the suitability of fresh diet (Sahayaraj and Jeyalekshimi, 2002; Domingues *et al.*, 2003).

Materials and methods

Stock culture

Adults of *M. sexmaculatus* (n = 40) were collected from the agricultural fields of Lucknow, India (26°50'N 80°54'E). Adults were fed with *ad libitum* supply of cowpea aphid, *Aphis craccivora* Koch (Hemiptera Aphididae) infested on *Vigna unguiculata* L. reared in a glasshouse (25 ± 2 °C, 65 ± 5% Relative Humidity). Adults were paired and placed in Petri dishes (hereafter, 9.0 × 2.0 cm), which were placed in Biochemical Oxygen Demand incubators (Yorco Super Deluxe, YSI-440, New Delhi, India) at 25 ± 1 °C, 65 ± 5% RH, 14L:10D photoperiod. Eggs laid were collected, and held in plastic Petri dishes until hatching, which usually occurs within 2-3 days. Once the first instars began moving on or away from the remnants of their egg clutch, they were removed using a fine camel hair paint brush from the original Petri dishes and assigned individually to clean experimental Petri dishes (size as above).

Collection of eggs used as a diet

Ten-day-old males and females (n = 30) were taken from stock culture, paired and allowed to mate. The females were isolated post mating and placed in Petri dishes (biotic and abiotic factors as above). Eggs laid were collected daily and used as a diet in the experiment.

Cannibalism of scattered and clustered eggs

To assess the effect of egg clustering pattern on consumption and development of *M. sexmaculatus*, first instar larva (from stock) was placed in a Petri dish and provided with either clustered (fresh or frozen) or an equal number of scattered eggs (fresh or frozen). In case of clustered pattern, eggs were placed in the centre of the Petri dish, while single eggs were placed randomly throughout the Petri dish. Fresh eggs were collected daily from an adult conspecific female, while frozen eggs were refrigerated for 24 hours prior to using them in the freezer (Godrej Eon, RT Eon 283 P 2.3 Refrigerator; temperature, −18 °C) to prevent its hatching. The number of eggs provided differed with instars: first instar 20 eggs, second instar 40 eggs, third instar 60 and fourth instar with 100 eggs.

The eggs consumed were recorded daily and the instars were provided with their pre-assigned diet every 24 hours. Percent consumption by each larval stage was calculated.

All larvae were examined daily so that all the developmental transitions could be recorded. Each of the four diet treatments *i.e.* clustered (fresh and frozen) and separated (fresh and frozen) was replicated 10 times.

Egg freshness and its effect on mating, development, reproduction, fitness and longevity

To assess the effect of egg freshness on mating and developmental parameters of *M. sexmaculatus*, first instar larvae were collected from stock and divided into two groups. In the first group, larvae were provided with a daily replenished diet of fresh conspecific eggs; number of eggs provided were different for each instar, as detailed above. In the second group, frozen conspecific eggs were provided as food to the larvae. All the eggs were provided in clustered form in the centre of the Petri dish and their position were marked. Each larva was reared individually in Petri dishes until adult emergence on their respective diets. Post-emergence, males and females were isolated and continued on their larval diet type; with 100 conspecific eggs provided daily per adult. Ten days post-emergence, males and females from each diet group were paired and allowed to mate. The females were isolated post mating and placed in Petri dishes (biotic and abiotic factors as above). The first batch of eggs laid as collected and held in Petri dishes until hatching. Each hatched larva (F_2 generation) was reared individually in Petri dishes and continued on the parental diet until adult emergence. Each larva was considered to be a replicate. Newly Emerged adults were reared on a similar diet and when ten days old, were paired in the following treatments: $♀_{\text{Fresh}} \times ♂_{\text{Fresh}}$ and $♀_{\text{Frozen}} \times ♂_{\text{Frozen}}$. The females were isolated post mating and were supplied with *ad libitum* eggs according to their diet groups. The position of the eggs supplied as diet was marked using a marker to distinguish them from the eggs laid by the females in both the diet groups. Eggs laid were collected every 24 hours by switching each female to clean Petri dish. The adults were fed their respective diets till their death for the assessment of longevity.

All the larvae in the above treatments were examined daily so that all the developmental transitions could be recorded. The mating parameters, *i.e.* time of commencement of mating (TCM; duration from the instant of cohabitation to intromission of aedeagus), latent period (LP; duration from intromission of aedeagus to first abdominal shaking) and copulation duration (CD; time from intromission until dismounting) of the pairs from above mating treatments were recorded. The fecundity (number of eggs laid) and percent egg viability (percentage of larvae emerged from total number of eggs laid) was recorded for 5 days after first oviposition by each female and longevity of adults was also recorded on both the diets.

Following McGraw and Caswell (1996), and Omkar and Mishra (2005), individual fitness (r) was also calculated as a performance measure using developmental time (D), survival (m = 0 or 1) and potential fecundity (V) using the following formula:

$$\text{Fitness (r)} = \frac{[\ln(\text{mV})]}{\text{D}}$$

Data analysis

Cannibalism of scattered and clustered eggs

To compare the effect of egg freshness and pattern on egg consumption, percent egg consumption and development duration of larval instars, data were subjected to two-way analysis of variance (ANOVA) with egg freshness and egg pattern as independent factors. The analysis was followed by a comparison of means using post hoc Tukey's HSD test at $\alpha = 0.05$. All statistical analyses were conducted using the MINITAB-16 statistical software (Minitab Inc., State College, PA, USA).

Egg freshness and its effect on mating, development, reproduction, fitness and longevity

To assess the effect of egg quality on the mating, development, fecundity, egg viability, fitness and longevity of adults, data were subjected to one-way ANOVA. The analysis was followed by a comparison of means using post hoc Tukey's HSD test at $\alpha = 0.05$. All statistical analyses were conducted using the MINITAB-16 statistical software.

Results

Cannibalism of scattered and clustered eggs

Two way ANOVA revealed significant effect of egg freshness ($F_{Freshness} = 6.77$, $P = 0.013$, df = 1,39) and pattern ($F_{Pattern} = 18.81$, $P = 0.000$, df = 1,39) along with their interaction ($F_{Freshness \times Pattern} = 91.05$, $P = 0.000$, df = 1,39) on egg consumption by first instar, with maximum consumption of frozen clustered eggs (figure 1). However results were found to be insignificant for second instar ($F_{Freshness} = 0.44$, $P = 0.510$, df = 1, 39; $F_{Pattern} = 2.77$, $P = 0.105$, df = 1,39; $F_{Freshness \times Pattern} = 1.00$, $P = 0.325$; df = 1,39). Third instar consumed maximum number of fresh eggs ($F_{Freshness} = 21.42$, $P = 0.000$, df = 1,39) in both clustered and scattered pattern ($F_{Pattern} = 0.00$, $P = 0.983$, df = 1,39), however, their interaction ($F_{Freshness \times Pattern} = 3.00$, $P = 0.092$, df = 1,39) was found to be insignificant. Reverse was observed for fourth instar, consumed maximum number of separated eggs ($F_{Pattern} = 7.02$, $P = 0.012$, df = 1,39) in both fresh and frozen condition ($F_{Freshness} = 1.88$, $P = 0.179$, df = 1,39), but their interaction was found to be insignificant ($F_{Freshness \times Pattern} = 1.41$, $P = 0.243$, df = 1,39).

Two way ANOVA on percent egg consumption revealed that the consumption by first instar was significantly influenced by both egg freshness and pattern; maximum percentage of frozen conspecific eggs were consumed in clustered pattern ($F = 23.05$, $P = 0.00$, df = 1,39) (figure 2). Results revealed insignificant effect of freshness and pattern on consumption by second instar ($F = 1.20$, $P = 0.280$, df = 1,39). Third instar larvae consumed the maximum of fresh conspecific eggs ($F = 14.91$, $P = 0.000$, df = 1,39) of both clustered and separated pattern ($F = 1.00$, $P = 0.323$, df = 1,39), however, interaction of egg freshness and pattern was found to be insignificant ($F = 0.93$, $P = 0.341$, df = 1,39). Egg freshness and pattern had insignificant effect on consumption by fourth instar ($F = 1.41$, $P = 0.243$, df = 1,39).

Developmental duration of immature stages was not influenced by both egg freshness and pattern (table 1).

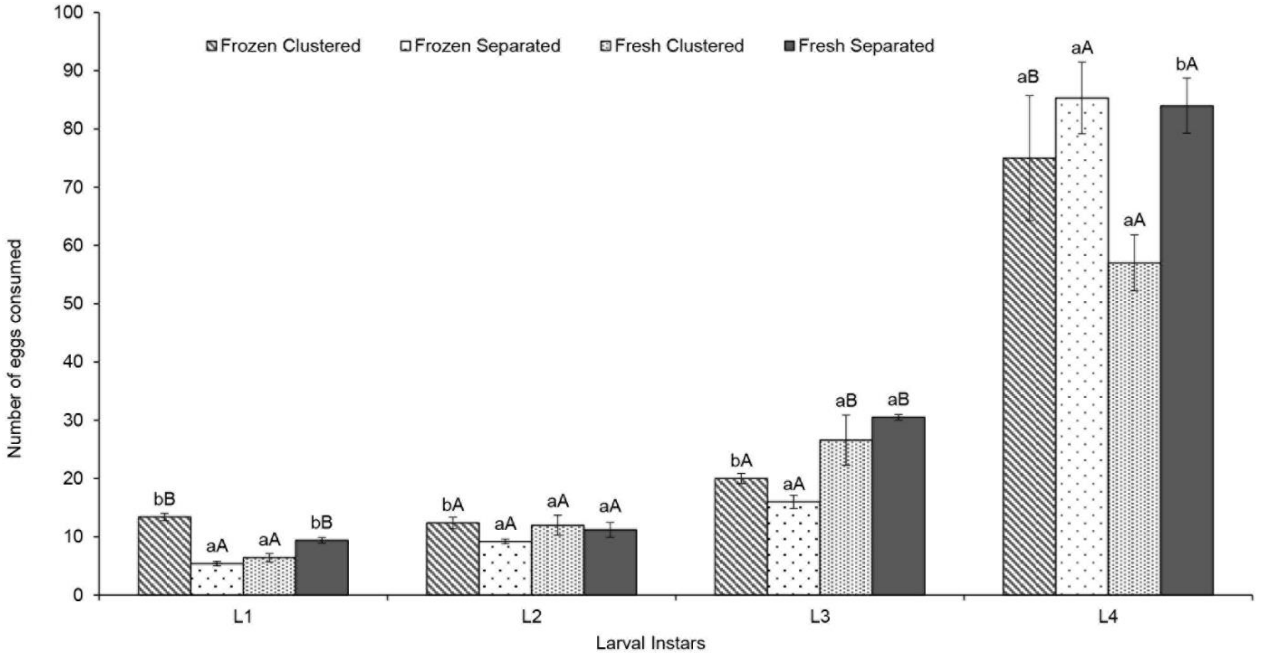

Figure 1. Number of eggs consumed by different larval instars when reared on equal number of clustered (fresh or frozen) and separated = scattered (fresh or frozen) conspecific eggs. Values are mean ± SE. Lowercase and uppercase letters indicate comparison of mean within diet treatments and among different larval instars respectively. Similar letters indicate lack of significant difference ($P > 0.05$).

Table 1. Effect of egg pattern along with egg freshness on the developmental duration of immature stages of F1 generation *M. sexmaculatus* under laboratory conditions.

Egg pattern	Egg freshness	Larval instars duration (days)					Pre-pupal stage (days)	Pupal stage (days)	Total duration (days)
		1st	2nd	3rd	4th	Total			
Clustered	Frozen	1.5aA ± 0.16	1.2aA ± 0.13	1.4aA ±0.16	2.3aA ± 0.26	6.4aA ± 0.34	1.15aA ± 0.21	3aA ± 0.14	12.55aA ± 0.43
Clustered	Fresh	1.4aA ± 0.22	1.5aA ± 0.22	1.2aA ± 0.13	2.6aA ± 0.16	6.9aA ± 0.27	1aB ± 0.25	2.7aA ± 0.26	12.2aA ± 0.46
Separated	Frozen	1.2aA ± 0.13	1.4aA ± 0.16	1.7bA ± 0.15	2.5aA ± 0.26	6.7bA ± 0.3	1.15aA ± 0.15	2.75aA ± 0.17	12.7aA ± 0.26
Separated	Fresh	1.4aA ± 0.22	1.5aA ± 0.22	1.2aA ± 0.13	2.6aA ± 0.16	6.0aA ± 0.29	1aA ± 0.25	2.7aA ± 0.26	12.2aA ± 0.46
$F_{\text{Egg Freshness}}$ (P-value)	df 1,39	0.09 (P > 0.05)	0.00 (P > 0.05)	8.23 (P < 0.05)	0.12 (P > 0.05)	0.97 (P > 0.05)	0.23 (P > 0.05)	0.02 (P > 0.05)	2.30 (P > 0.05)
$F_{\text{Egg Pattern}}$ (P-value)	df 1,39	2.23 (P > 0.05)	0.38 (P > 0.05)	0.51 (P > 0.05)	1.99 (P > 0.05)	0.11 (P > 0.05)	2.87 (P > 0.05)	0.02 (P > 0.05)	0.04 (P > 0.05)
$F_{\text{Egg Freshness} \times \text{Pattern}}$ (P > 0.05)	df 1,39	0.09 (P > 0.05)	3.45 (P > 0.05)	2.06 (P > 0.05)	0.50 (P > 0.05)	3.88 (P > 0.05)	2.87 (P > 0.05)	2.14 (P > 0.05)	0.35 (P > 0.05)

Values are mean ± SE. Lowercase and uppercase letters indicates comparison of mean within and between treatments respectively. Similar letters indicate lack of significant difference (P > 0.05).

Table 2. Duration of the developmental stages of F2 generation of *M. sexmaculatus* reared on fresh and frozen clustered conspecific eggs under laboratory conditions.

Egg freshness		Incubation period (days)	Larval instar duration (days)					Pre-pupal stage (days)	Pupal stage (days)	Total development duration (days)
			1st	2nd	3rd	4th	Total			
Frozen		2.71b ± 0.18	1.85a ± 0.14	1.57a ± 0.20	1.85a ± 0.40	3.28a ± 0.47	8.71a ± 0.18	0.92a ± 0.07	2.78a ± 0.26	15.14a ± 0.40
Fresh		1.8b ± 0.2	2.2a ± 0.20	1.2a ± 0.20	1.6a ± 0.24	3.2a ± 0.20	8.2a ± 0.37	0.8a ± 1.22	3.2a ± 0.12	14.2a ± 0.37
$F_{\text{Egg Freshness}}$ (P-value)	df 1,11	10.94 (P < 0.05)	2.07 (P > 0.05)	1.60 (P > 0.05)	0.24 (P > 0.05)	0.02 (P > 0.05)	1.82 (P > 0.05)	0.94 (P > 0.05)	1.55 (P > 0.05)	2.68 (P > 0.05)

Values are mean ± SE. Lowercase letters indicate comparison of mean between treatments. Similar letters indicate lack of significant difference (P > 0.05).

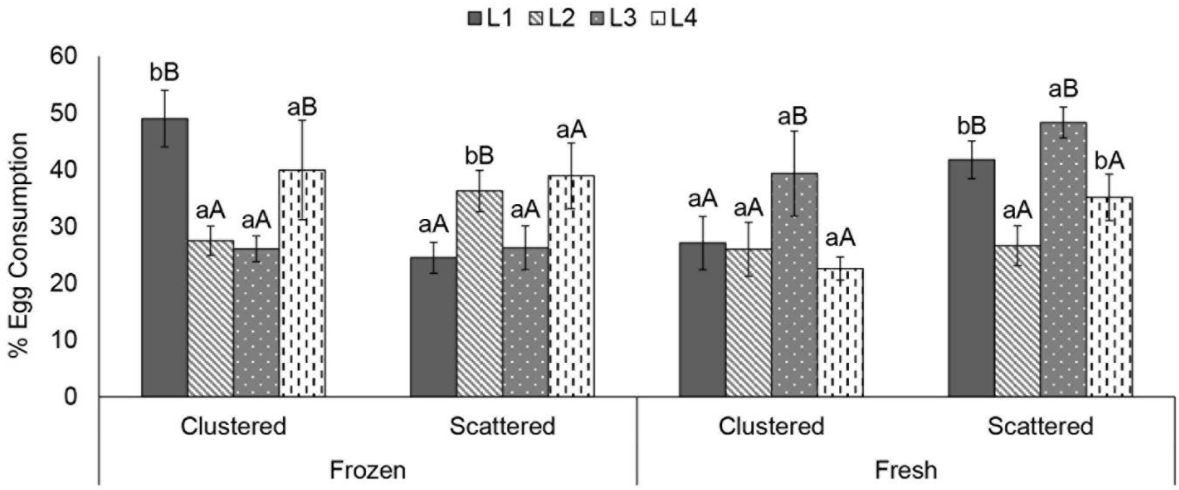

Figure 2. Effect of egg freshness and pattern on percent egg consumption by larval instars of *M. sexmaculatus.* Values are mean ± SE. Lowercase and uppercase letters indicate comparison of mean within and between treatments. Similar letters indicate lack of significant difference (P > 0.05).

Effect of egg freshness on mating, development, reproduction, fitness and longevity

The results of one-way ANOVA revealed that total developmental duration of larval instars was not affected by the freshness of eggs provided even when their parents had been reared in larval and adult stages on the same diet (table 2). The resultant adults also did not show any significant variation in mating parameters, *i.e.* time to commencement of mating (TCM) (F = 3.87, P = 0.069, df = 1,15), latent period (LP) (F = 0.13, P = 0.724, df = 1,15) and copulation duration (CD) (F = 0.04, P = 0.840, df = 1,15) (figure 3). However, fecundity, percent egg viability, and longevity were significantly influenced by egg freshness. The females reared on frozen conspecific eggs had higher fecundity (F = 16.69, P = 0.001, df = 1,15) and percent egg viability (F = 26.49, P = 0.000, df = 1,15) (figure 4) but shorter lifespan as compared to those reared on fresh conspecific eggs. Females, in general, lived significantly longer than the males (F_{Female} = 21.54, P = 0.000, df = 1,15; F_{Male} = 59.70, P = 0.000, df = 1,15) on both diets (figure 5). Fitness values, however, did not differ significantly with egg freshness (F = 0.01, P = 0.907, df = 1,15) (figure 6).

Discussion

The present study revealed that *M. sexmaculatus* is able to complete its reproduction and development on an exclusive diet of fresh as well as frozen conspecific eggs. Neither pattern (clustered or scattered) nor did the egg freshness had a significant effect on total consumption by immature stages. However, percent egg consumption of frozen clustered eggs was significantly higher by first instars and of fresh scattered eggs by fourth instars. The developmental duration of immature stages and mating parameters of adults (TCM, LP, and CD) were found to be similar on both diets, however, fecundity and percent egg viability were more on frozen conspecific eggs; longevity followed the reverse trend.

Egg freshness and pattern had no significant effect on consumption by larval instars. Among developing larval instars, fourth instar larvae consumed the maximum number of eggs (fresh or frozen) as has been reported earlier (*Coccinella septempunctata* L.) (Omkar and Srivastava, 2001; 2003; Hodek *et al.*, 2012; Kumar *et al.*, 2014) as the fourth instar larvae require more energy for pupation and metamorphosis (Omkar and Srivastava, 2001).

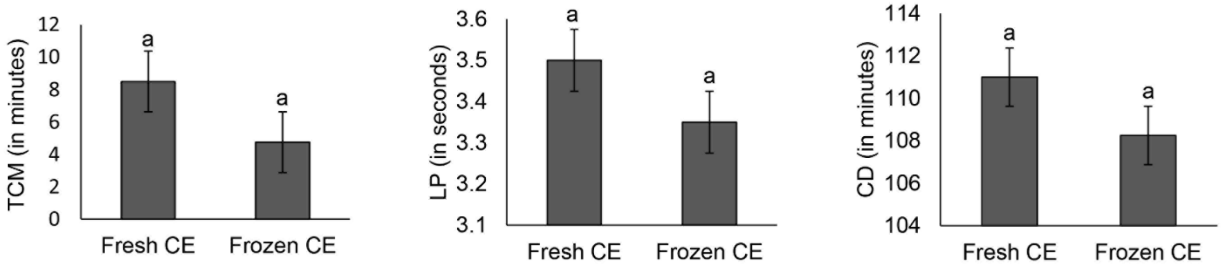

Figure 3. Effect of egg freshness on mating parameters: TCM (Time to Commencement of Mating), LP (Latent Period) and CD (Copulation Duration) of *M. sexmaculatus.* Values are mean ± SE. Lowercase letters indicate comparison of mean within treatments. Similar letters indicate lack of significant difference (P > 0.05). (CE = Conspecific Eggs).

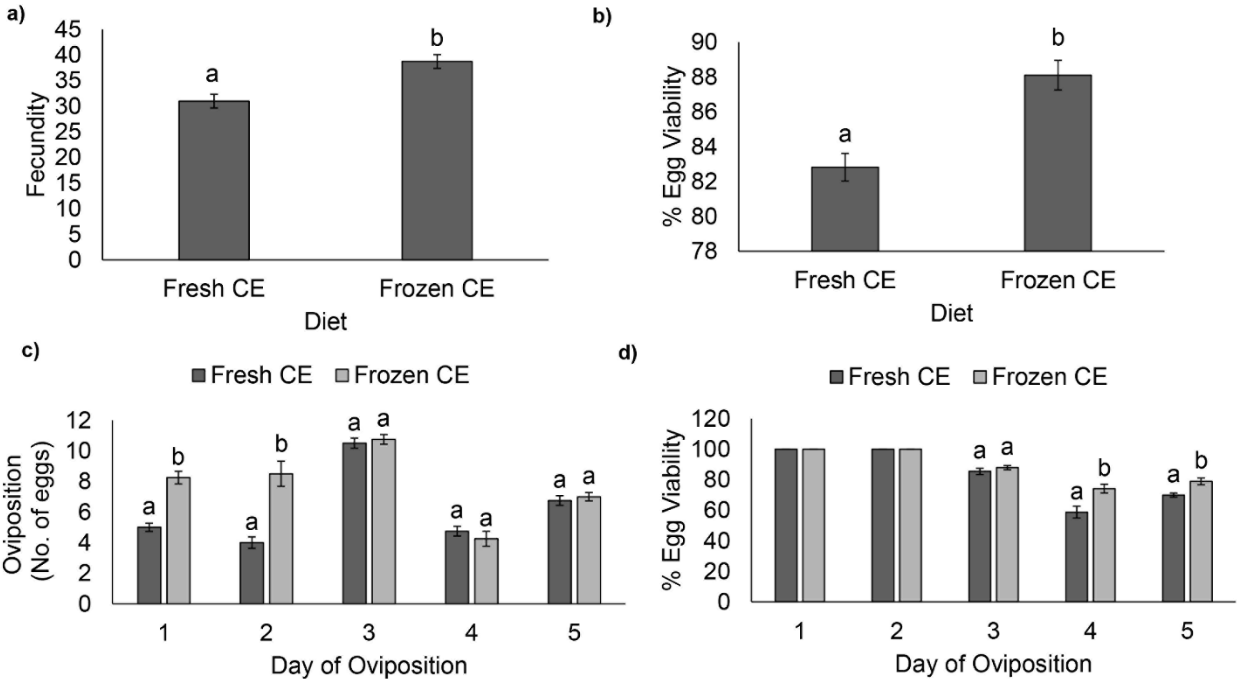

Figure 4. Effect of diet on (a) fecundity, (b) percent egg viability, (c) oviposition and (d) percent egg viability per day of *M. sexmaculatus.* Values are mean ± SE. Lowercase letters indicate the comparison of means within diet treatments. Similar letters indicate lack of significant difference (P value > 0.05). (CE = Conspecific Eggs).

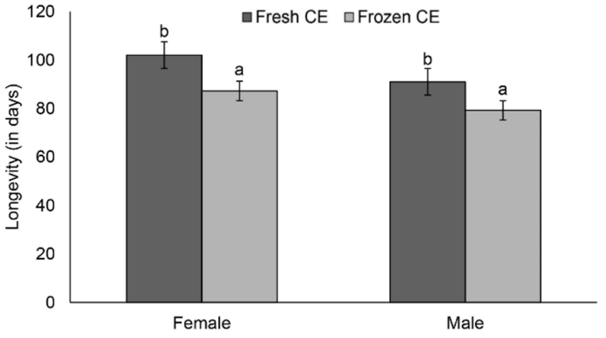

Figure 5. Effect of egg freshness on longevity of *M. sexmaculatus.* Values are mean ± SE. Lowercase letters indicate the comparison of means within diet treatments. Similar letters indicate lack of significant difference (P > 0.05). (CE = Conspecific Eggs).

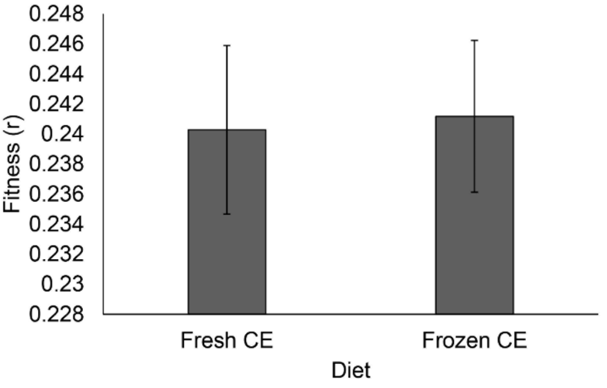

Figure 6. Effect of egg freshness on fitness of *M. sexmaculatus.* Values are mean ± SE. (CE = Conspecific Eggs).

Clustered and scattered eggs were eaten in similar amounts by *M. sexmaculatus* larvae, which was contrary to our hypothesis. Agarwala and Dixon (1993) have also reported that the incidence of cannibalism is similar in singly laid as well as clustered eggs. However, they have found that predatory attempts by heterospecifics were more likely on single eggs than clustered ones. The clustering of eggs works as a defensive mechanism against intraguild predation rather than cannibalism has been strongly suggested (Mueller *et al.*, 1984; Agarwala *et al.*, 1998). Studies on ladybirds, *Cycloneda sanguinea* (L.), *Harmonia axyridis* (Pallas) and *Olla v-nigrum* (Mulsant) have however revealed that the probability of cannibalism was higher for clustered eggs as compared to single eggs, which is probably because in nature the chances of an encounter with clustered eggs are more likely (Michaud and Grant, 2004).

The percent egg consumption at each larval stage revealed that the egg freshness did have a significant effect on the preference of pattern by first and fourth instars. First instars preference for frozen clustered and fourth instars for fresh scattered eggs, probably indicates, that either (a) younger instars find clusters easier to find and consume than later instars, or (b) the nutritional content of the eggs differ with freshness and thus their suitability differs for each instar. However, the latter reason is not supported in studies on brown marmorated stink bug, *Halyomorpha halys* (Stal) (Skillman and Lee, 2017), where there was no alteration in the nutrient content of the eggs when frozen within 1 or 3 days of oviposition. However, for confirmation, nutritional analysis of eggs is required. The attractiveness of the clustered eggs to the first instars resulting in increased percent consumption may be attributed to their limited mobility immediate after hatching (Agarwala and Dixon, 1993).

Mating parameters, *i.e.* TCM, LP and CD, were not affected by the freshness of the egg diet and were statistically similar on frozen eggs, which suggests similar nutritional content of the eggs. Nutritional status of adults is known to directly influence mating and reproductive parameters (Aluja *et al.*, 2001; Fricke *et al.*, 2008). However, in wolf spider, *Pardosa milvina* (Hentz), the quality of diet has no effect on time to commence mating and mating duration (Wilder and Rypstra, 2008).

Fecundity and percent egg viability was higher on frozen eggs as compared to fresh eggs. Studies in cuttlefish, *Sepia officinalis* (L.) (Domingues *et al.*, 2003) demonstrated higher fecundity on frozen shrimp, *Palaemonetes varians* (Leach). On the contrary, a study by Abdel-Salam and Abdel-Baky (2001) on *H. axyridis* demonstrated that females were more fecund on *Sitotroga cerealella* (Olivier) fresh eggs as compared to frozen ones. Several studies using different species of algae have concluded that frozen algae can be a suitable substitute for fresh algae (Baer and Goulden, 1998). Cotelle and Ferard (1996) reported significant differences in fecundity of *Daphnia magna* Straus on frozen algae (*Raphidocelis subcapitata* Kors.) at different temperatures *i.e.* −20, −80 and −196 °C compared with fresh algae with highest fecundity on fresh diet followed by frozen diet (−80 °C). As fecundity cannot be used as an only factor for determining the performance of the beetle, we also calculated individual fitness (r) integrating the fecundity along with survival and developmental duration (McGraw and Caswell, 1996; Omkar and Mishra, 2005), the results of which were similar on both the diets indicating suitability and similar nutritional profile of both the diets. This also went against our hypothesis that fresh eggs would be better suited for life history traits than frozen eggs.

Freshness of eggs, did however have a significant effect on the longevity of adults. Adults reared on fresh conspecific eggs tend to live longer than those reared on frozen conspecific eggs. It is possible that transgenerational rearing on a specific diet, may exacerbate/ improve the insignificant nutritional changes, which may be confirmed by biochemical analysis (Domingues *et al.*, 2003; Andersen, 2012; Skillman and Lee, 2017) of fresh and frozen eggs. Sahayaraj and Jeyalekshmi (2002) reported that adults of *Rhynocoris marginatus* (F.) tended to live longer on live *Corcyra cephalonica* (Stainton) larvae compared to frozen ones.

Freshness of egg or difference in availability pattern had no significant effect on immature development of *M. sexmaculatus* attributing to insignificant changes in the nutritional value of the frozen diet. Studies in predatory stinkbug *Eocanthecona furcellata* (Wolff) also reported insignificant difference in development of adults on live and frozen larvae of *Spodoptera litura* (F.) (Yasuda and Wakamura, 1992). Thus frozen diet can be used for rearing under laboratory conditions when aphid density is low.

In conclusion, our study demonstrated: (i) no preference for clustered eggs over scattered eggs; total consumption by immature instars were similar on both diets indicating that the clustering of eggs does not outweigh the likelihood of cannibalism, (ii) however, percent consumption of clustered (fresh or frozen) and separated (fresh or frozen) differed with instars, (iii) egg freshness did not affect mating and reproductive parameters while, fecundity and percent egg viability increased on frozen eggs. Longevity followed the reverse trend, (iv) immature development was not affected by both egg pattern and egg freshness, and (v) the overall performance of beetle was similar on both diets. Since aphidophagous ladybird *M. sexmaculatus* utilizes frozen conspecific eggs for growth, development, and reproduction as on the fresh conspecific eggs, the frozen eggs can be used as an alternative diet under laboratory conditions.

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References

ABDEL-SALAM A. H., ABDEL-BAKY N. F., 2001.- Life table and biological studies of *Harmonia axyridis* Pallas (Col., Coccinellidae) reared on the grain moth eggs of *Sitotroga cerealella* Olivier (Lep., Gelechiidae).- *Journal of Applied Entomology*, 125 (8): 455-462.

AGARWALA B. K., DIXON A. F. G., 1992.- Laboratory study of cannibalism and interspecific predation in ladybirds.- *Ecological Entomology*, 17 (4): 303-309.

AGARWALA B. K., DIXON A. F. G., 1993.- Why do ladybirds lay eggs in clusters?- *Functional Ecology*, 7: 541-548.

AGARWALA B. K., BHATTACHARYA S., BARDHANROY P., 1998.- Who eats whose eggs? Intra-versus inter-specific interactions in starving ladybird beetles predaceous on aphids.- *Ethology Ecology & Evolution*, 10 (4): 361-368.

AHMAD M., CHAUDHARY S. U., AFZAL A. J., TARIQ M., 2015.- Starvation-induced dietary behaviour in *Drosophila melanogaster* larvae and adults.- *Scientific Reports*, 5: 14285.

ALUJA M., JACOME I., MACIAS-ORDONEZ R., 2001.- Effect of adult nutrition on male sexual performance in four neotropical fruit fly species of the genus *Anastrepha* (Diptera: Tephritidae).- *Journal of Insect Behavior*, 14 (6): 759-775.

ANDERSEN N. G., 2012.- Influences of potential predictor variables on gastric evacuation in Atlantic cod *Gadus morhua* feeding on fish prey: parameterization of a generic model.- *Journal of Fish Biology*, 80 (3): 595-612.

AYASSE M., PAXTON R., 2002.- Brood protection in social insects, pp. 117-148. In: *Chemoecology of insect eggs and egg deposition* (HILKAR M., MEINERS T. Eds).- Blackwell, Berlin, Germany.

BAER K. N., GOULDEN C. E., 1998.- Evaluation of a high-hardness COMBO medium and frozen algae for *Daphnia magna*.- *Ecotoxicology and Environmental Safety*, 39 (3): 201-206.

CAZZANIGA N. J., 1990.- Predation of *Pomacea canaliculata* (Ampullariidae) on adult *Biomphalaria peregrina* (Planorbidae).- *Annals of Tropical Medicine & Parasitology*, 84 (1): 97-100.

CLAESSEN D., DE ROOS A. M., PERSSON L., 2004.- Population dynamic theory of size-dependent cannibalism.- *Proceedings of the Royal Society of London B: Biological Sciences*, 271 (1537): 333-340.

CASTRO-GUEDES C. F., ALMEIDA L. M., PENTEADO S. R. C., MOURA M. O., 2016.- Effect of different diets on biology, reproductive variables and life and fertility tables of *Harmonia axyridis* (Pallas) (Coleoptera, Coccinellidae).- *Revista Brasileira de Entomologia*, 60 (3): 260-266.

COTELLE S., FERARD J. F., 1996.- Effects of algae frozen at different temperatures on chronic assessment endpoints observed with *Daphnia magna*.- *Ecotoxicology and Environmental Safety*, 33 (2): 137-142.

DEVEE A., ARVANITI K., PERDIKIS D., 2018.- Intraguild predation among three aphidophagous predators.- *Bulletin of Insectology*, 71 (1): 11-19.

DIXON A. F. G., 2000.- *Insect predator-prey dynamics: ladybird beetles and biological control*.- Cambridge University Press, Cambridge, UK.

DIXON A. F. G., AGARWALA B. K., 2002.- Triangular fecundity function and aging in ladybird beetles.- *Ecological Entomology*, 27 (4): 433-440.

DOMINGUES P., SYKES A., SOMMERFIELD A., ANDRADE J. P., 2003.- Effects of feeding live or frozen prey on growth, survival and the life cycle of the cuttlefish, *Sepia officinalis* (Linnaeus, 1758).- *Aquaculture International*, 11 (5): 397-410.

DONG Q., POLIS G. A., 1992.- The dynamics of cannibalistic populations: a foraging perspective, pp. 13-37. In: *Cannibalism: ecology and evolution among diverse taxa* (ELGAR M. A., CRESPI B. J., Eds).- Oxford University Press, New York, USA.

FELIX S., SOARES A. O., 2004.- Intraguild predation between the aphidophagous ladybird beetles *Harmonia axyridis* and *Coccinella undecimpunctata* (Coleoptera: Coccinellidae): the role of body weight.- *European Journal of Entomology*, 101: 237-242.

FOX L. R., 1975.- Cannibalism in natural populations.- *Annual Review of Ecology and Systematics*, 6: 87-106.

FRICKE C., BRETMAN A., CHAPMAN T., 2008.- Adult male nutrition and reproductive success in *Drosophila melanogaster*.- *Evolution: International Journal of Organic Evolution*, 62 (12): 3170-3177.

HAMASAKI K., MATSUI M., 2006.- Development and reproduction of an aphidophagous coccinellid, *Propylea japonica* (Thunberg) (Coleoptera Coccinellidae), reared on an alternative diet, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs.- *Applied Entomology and Zoology*, 41 (2): 233-237.

HO F. K., DAWSON P. S., 1966.- Egg cannibalism by *Tribolium* larvae.- *Ecology*, 47 (2): 318-322.

HODEK I., VAN EMDEN H. F., HONEK A., 2012.- *Ecology and behaviour of ladybird (Coccinellidae)*.- John Wiley and Sons, Oxford, UK.

JOSEPH S. B., SNYDER W. E., MOORE A. J., 1999.- Cannibalizing *Harmonia axyridis* (Coleoptera: Coccinellidae) larvae use endogenous cues to avoid eating relatives.- *Journal of Evolutionary Biology*, 12 (4): 792-797.

KANG Y., RODRIGUEZ-RODRIGUEZ M., EVILSIZOR S., 2015.- Ecological and evolutionary dynamics of two-stage models of social insects with egg cannibalism.- *Journal of Mathematical Analysis and Applications*, 430 (1): 324-353.

KUMAR B., BISTA M., MISHRA G., 2014.- Stage specific consumption and utilization of aphids, conspecific and heterospecific eggs by two species of *Coccinella* (Coleoptera: Coccinellidae).- *European Journal of Entomology*, 111 (3): 363-369.

MCGRAW J. B., CASWELL H., 1996.- Estimation of individual fitness from life-history data.- *The American Naturalist*, 147 (1): 47-64.

MICHAUD J. P., 2003.- A comparative study of larval cannibalism in three species of ladybird.- *Ecological Entomology*, 28: 92-101

MICHAUD J. P., GRANT A. K., 2004.- Adaptive significance of sibling egg cannibalism in Coccinellidae: comparative evidence from three species.- *Annals of the Entomological Society of America*, 97 (4): 710-719.

MUELLER R. H., THOMSON M. E., DIPARDO R. M., 1984.- Stereo- and regioselective total synthesis of the hydropyrido [2,1,6-*de*]quinolizine ladybug defensive alkaloids.- *The Journal of Organic Chemistry*, 49: 2217-2231.

OMKAR, BIND R. B., 2004.- Prey quality dependent growth, development and reproduction of a biocontrol agent, *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae).- *Biocontrol Science and Technology*, 14 (7): 665-673.

OMKAR, MISHRA G., 2005.- Preference-performance of a generalist predatory ladybird: a laboratory study.- *Biological Control*, 34 (2): 187-195.

OMKAR, SRIVASTAVA S., 2001.- Comparative predatory potential of a ladybird beetle, *Coccinella septempunctata* Linn. on six prey species.- *Biological Memoirs*, 27: 59-63.

OMKAR, SRIVASTAVA S., 2003.- Functional response of the seven-spotted ladybeetle, *Coccinella septempunctata* Linnaeus on the mustard aphid, *Lipaphis erysimi* (Kaltenbach).- *Insect Science and its Applications*, 23 (2): 149-152.

OMKAR, PERVEZ A., GUPTA A. K., 2004.- Role of surface chemicals in egg cannibalism and intraguild predation by neonates of two aphidophagous ladybirds, *Propylea dissecta* and *Coccinella transversalis*.- *Journal of Applied Entomology*, 128 (9-10): 691-695.

OMKAR, PERVEZ A., GUPTA A. K., 2006.- Why do neonates of aphidophagous ladybirds preferentially consume conspecifics eggs in presence of aphids?- *Biocontrol Science and Technology*, 16 (3): 233-243.

OMKAR, PERVEZ A., MISHRA G., SRIVASTAVA S., SINGH S. K., GUPTA A. K., 2005.- Intrinsic advantages of *Cheilomenes sexmaculata* over two coexisting *Coccinella* species (Coleoptera: Coccinellidae).- *Insect Science*, 12 (3): 179-184.

OSAWA N., 1993.- Population field studies of the aphidophagous ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae): life tables and key factor analysis.- *Researches on Population Ecology*, 35 (2): 335-348.

PERVEZ A., GUPTA A. K., OMKAR, 2006.- Larval cannibalism in aphidophagous ladybirds: influencing factors, benefits and costs.- *Biological Control*, 38 (3): 307-313.

PERVEZ A., GUPTA A. K., OMKAR, 2013.- Kin recognition and avoidance of kin cannibalism by the larvae of co-occurring ladybirds: a laboratory study.- *European Journal of Entomology*, 102(3): 513-518.

PFENNIG D. W. 1997.- Kinship and cannibalism.- *Bioscience*, 47: 667-675.

PFENNIG D. W., HO S. G., HOFFMAN E. A., 1998.- Pathogen transmission as a selective force against cannibalism.- *Animal Behaviour*, 55 (5): 1255-1261.

RUDOLF V. H., ANTONOVICS J., 2007.- Disease transmission by cannibalism: rare event or common occurrence?- *Proceedings of Royal Society London B Biological Sciences*, 274: 1205-1210.

SAHAYARAJ K., JEYALEKSHIMI T., 2002.- Mass rearing of *Rhynocoris marginatus* Fab on live and frozen larvae of *Corcyra cephalonica* biology.- *Entomologica Croatica*, 6 (1-2): 35-49.

SANTI F., MAINI S., 2007.- Ladybirds mothers eating their eggs: is it cannibalism?- *Bulletin of Insectology*, 60 (1): 89-91.

SANTI F., BURGIO G., MAINI S., 2003.- Intra-guild predation and cannibalism of *Harmonia axyridis* and *Adalia bipunctata* in choice conditions.- *Bulletin of Insectology*, 56 (2): 207-210.

SCHAUSBERGER P, CROFT B. A., 2001.- Kin recognition and larval cannibalism by adult females in specialist predaceous mites.- *Animal Behaviour*, 61: 459-464

SKILLMAN V. P., LEE J. C., 2017.- Nutrient content of brown marmorated stink bug eggs and comparisons between experimental uses.- *Journal of Insect Science*, 17 (6): 120.

STUBBS M., 1980.- Another look at prey detection by coccinellids.- *Ecological Entomology*, 5 (2): 179-182.

TRUMBO S. T., VALLETTA R. C., 2007.- The costs of confronting infanticidal intruders in a burying beetle.- *Ethology*, 113 (4): 386-393.

WILDER S. M., RYPSTRA A. L., 2008.- Diet quality affects mating behaviour and egg production in a wolf spider.- *Animal Behaviour*, 76 (2): 439-445.

WU L., ZHANG H., HE T., LIU Z., PENG Y., 2013.- Factors influencing sexual cannibalism and its benefit to fecundity and offspring survival in the wolf spider *Pardosa pseudoannulata* (Araneae: Lycosidae).- *Behavioural Ecology and Sociobiology*, 67: 205-212

YASUDA T., WAKAMURA S., 1992.- Rearing of the predatory stink bug, *Eocanthecona furcellata* (Wolff) (Heteroptera: Pentatomidae), on frozen larvae of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae).- *Applied Entomology and Zoology*, 27 (2): 303-305.

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