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Development and Growth of *Exochomus quadripustulatus* (Coleoptera: Coccinellidae): a Predator of Mussel Scale *Lepidosaphes ulmi* (Homoptera: Diaspididae) on Apple

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Abstract.- *Exochomus quadripustulatus* L., a polyphagous predatory beetle of both aphids and diaspidids, is capable of developing on mussel scale *Lepidosaphes ulmi* L. alone. The development of *E. quadripustulatus* from egg to pupa was completed in 94-128 (107.5 ± 8.35) and 88-112 (97.83 ± 3.8) days in male and female, respectively. Adult female has a unique tendency of egg laying with varying numbers of eggs/day under protected places; technique of egg collection is described. A significant linear relationship on the biological measurement was found between head capsule width and the larval body length; size and weight of both larva and adult. Some of the adults *E. quadripustulatus* when fed on *L. ulmi* lived for 114-305 days while other survived 8-53 days under field insectary conditions. It may be concluded that *E. quadripustulatus* is capable of playing its role in reducing the mussel scale *L. ulmi* populations on apple alone even if the other alternate prey (aphid) is not available in the field.

Key Words: *Exochomus quadripustulatus, Lepidosaphes ulmi*, apple, coccinellids, mussel scale.

INTRODUCTION

Coccinellids are probably the best studied natural enemies perhaps due to the harmful side effects of toxic chemicals on crops and their potential of being biocontrol agents of aphids and coccids (Hodek, 1967; Hagen, 1974; Henderson and Albrecht, 1988*b*). They constitute an important portion of the fauna of horticultural crops all over the world. Most predatory species are either considered as aphidophagous (*e.g*., *Coccinella septempunctata* L.) or coccidophagous *e.g.*, *Chilocorus nigritus* (F.), *Diploponis inconspicuus* Pope both feed on citrus scale, while some species are both coccido-and aphidophagous (Brettel, 1964; Radwan and Lövei, 1983; Samway and Wilson, 1988; Lövei *et al*., 1991). The four-spotted ladybeetle *Exochomus quadripustulatus* L. is considered as an important polyphagous predator of both aphids and scale insects. Despite the fact that many researchers have studied various *Exochomus* species as predators of different prey on horticultural crops (Smith and Coppel, 1957; Meriacker *et al*., 1990; Umeh, 1990; Cudjoe *et al*., 1992; Kanika-Kiamfu *et al*., 1993), information is lacking on *E. quadripustulatus* as a predator of scale insects. Its distribution and occurrence in the presence of either scales or aphids, effect of chemicals (Tondeur *et al*., 1993), rearing on artificial diet (Henderson and Albrecht, 1988*a*), cytological species-separation (Smith, 1965), determination of sex ratio (Henderson and Albrecht, 1988b), feeding on aphids (Radwan and Lövei, 1982, 1983) and larval biology on citrus scale, *Planococcus citri* Risso (Uygun, 1978) have been studied, but information on its predation of scale insects infesting apple is not available.

The increasing interest and the impotance of polyphagous predators in general (Luff, 1983; Tolonen, 1995) and *Exochomus* spp in particular (Eschmatov and Saidova, 1993) for natural control has been realised in recent decades in order to overcome environmental contamination being caused by chemicals. Tondeur *et al*. (1993), who studied the impact of amitraz (MITAC) on *E. quadripustulatus* emphasised that this ladybird, a major predator of coccids, justifies its protection when pest management is carried out.

Although some biological parameters of *E.*

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quadripustulatus as predator of *Pulvinaria reglis* Canard (Homoptra: Coccidae) have been studied in Germany by Sengonca and Arnold (2003), no investigations on the development and growth of *E. quadripustulatus* with particular reference to *L. ulmi* on apple have been done so far. Therefore, the present study determines the ability of this predator to develop under field insectary conditions in north-east England.

MATERIALS AND METHODS

Oviposition, incubation period and hatchability of eggs

Samples of adult *E. quadripustulatus* were collected from apple trees previously planted at the University of Newcastle Field Laboratory at Heddon-on-the-Wall. They were placed in pairs (one male, one female) in separate transparent plastic containers (5 cm in diameter, 3 cm in depth) with a lid on the top having gauze-covered window (2 x 2 cm) to allow for fresh air exchange and offered mussel scale (*L. ulmi*) on 21 March 1994. Initially five pairs of beetles were used for egg laying but later, when more were available in the field, the numbers of pairs were increased to 20. One to three pieces (4 to 5 cm in length) of scale-infested spurs were provided to each pair. Food was replaced after every two days till the female stopped egg laying or died. In each cage, a piece of folded paper strip and rolled toilet tissue paper loosely fitted to the circular hole in the container lid was used as a site for egg laying. Observations on egg laying and collection were made once or twice every day.

Incubation period was determined by the duration (in days) from egg laying until the hatching of the last larva. Hatchability (excluding mechanically damaged but including cannibalised or desiccated eggs) was expressed as the percentage of the larvae that hatched and the eggs observed.

Rearing of immature stages

From a single batch of eggs (excepting a single larva taken from an earlier batch) laid on 27 April 1994 and hatched between 16-18 May 1994, 12 newly emerged first instar larvae were confined individually in glass tubes (approximately 2.5 cm in diameter and 5 cm in depth) because of their small size and delicacy. They were provided with unknown but ample numbers of mixed stages of *L. ulmi*, such as gravid females carefully removed from the infested spur and ventrally exposed, or first instar larvae, the only available stages of prey in the field at this time of experiment. The larvae of *E. quadripustulatus* were transferred to larger containers soon after moulting and entering into second instar, till the final adult stage was reached. Similarly, food was again provided as whole spurs as described above. Observations were facilitated with the aid of a stereomicroscope with an eyepiece graticule.

Samples representing the different stages of the predator were preserved for measuring after each moult. Measurements of the length of the larval specimens were based on the longitudinal distance from the anterior margin of the head to the posterior margin of the last abdominal segment (while resting) and the breadth of the head capsule across the distance of the two eyes. Measurements were taken within 24h of moulting. Similarly, the pupal length and breadth were also recorded. Live body weight of each larval instar at each moulting stage was carefully observed and recorded to a precision of 0.1μg on a CAHN-automatic electro-microbalance.

Post development parameters

Four males and six females of newly emerged *E. quadripustulatus* adults were placed separately in the container and offered unknown numbers of prey (virgin or gravid females) in the same manner as described above. The fresh body weight, size (length and breadth) of all adults were measured soon after or within 24h of emergence from pupa; and the adult longevity was also recorded.

RESULTS

Oviposition

Of the 20 pairs of adult *E. quadripustulatus*, only 10 female adults actually oviposited (Table I). The first egg was laid on 23 March whereas regular egg laying started from the first week of April to the last of May 1994; the last egg was recorded on 26 May 1994. Almost all the eggs were deposited either in groups (2-16) or singly, surprisingly, inside the layers of folded tissue paper which was previously fitted to the circular hole made in the lid of the container. No eggs were found either on the scale-infested spur or on the folded paper. Occasionally (once or twice), eggs were collected from the wall of the container and once a batch of 16 eggs was laid on the cotton ball initially placed in the container to observing the egg laying site in the confined cage. The maximum egg-laying period, i.e., from the day the first eggs were deposited until the day the female deposited the last eggs, was 43 days. During this period 72 eggs were laid by a single female with an average of 1.67 per day. Approximately, the same average (1.64/day) was resulted from the minimum egg-laying period of 11 days during which the female laid 18 eggs. The highest number of eggs deposited by a female in one day was 16 while the lowest was one.

Table I.- **Fecundity and hatchability of *Exochomus quadripustulatus* with *Lepidosaphes ulmi* as prey in field insectary under ambient conditions.**

Predato r Code Number	Eggs laid			Eggs damaged				Numbers of eggs hatched	% hatched**
	Total eggs laid	Duration of eggs laying (d)*	Average ± SE per day	By handling	Canni-balised	Desiccate d	Total		
1	72	43	1.67± 0.58	10	5	5	20	52	83.87
2	20	13	1.54 ± 0.77	1	3	1	5	15	78.95
3	18	11	1.64 ± 0.89	3	2	-	5	13	86.67
4	18	36	0.50 ± 0.29	3	1	-	4	14	93.33
5	17	20	0.85 ± 0.67	-	3	-	3	14	82.35
6	5	1	5.00	1	-	-	1	4	Too few
7	4	1	4.00	-	-	-	-	4	eggs
8	2	1	2.00	1	-	-	1	1	"
9	1	1	1.00	-	-	-		1	"
10	2	1	2.00	-	-	2	2	0	"

*d, days; From first to last day of egg laying; **Excluding eggs damaged during handling

Egg hatching

Most of the eggs hatched between 27-32 days while the peak numbers were found between 31-32 days (Fig. 1). The longest duration of egg development was 49 days and the shortest 13 days. The eggs laid in early spring (first week of April) took longer time to incubate than those laid in summer (late May). The hatching percentage varied from 78-93% excluding batches too small to calculate a meaningful percentage except for two eggs which failed to hatch due to unknown reasons (Table I). Of 115 eggs, most of these (60.8%) hatched in ≥4-5 weeks under ambient conditions while others hatched in ≤2 weeks which suggests that incubation is temperature dependent.

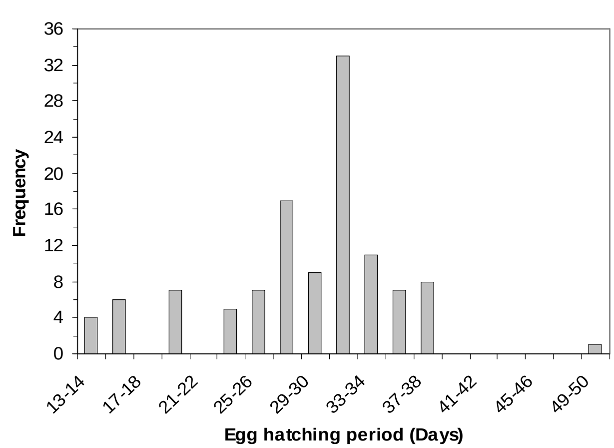

Fig. 1. Frequency distribution of egg hatching period (days) of *Exochomus quadripustulatus* L. under ambient conditions.

Development of immature stages

Larvae

The mean developmental periods of all four larval instars were 55±3.97 and 50.2±2.39 days for male and female larvae of *E. quadripustulatus*, respectively (Table II). The larval duration in fourth instar was longer than the second instar. Development of larvae took longer than other immature stages whereas the shortest development was observed at the prepupal stage. Analysis of variance (general linear model) showed a highly significant difference between all developmental stages ($F^6{}_{70}$=193.21, P<0.001) and as well as a significant ($F^1{}_{70}$=5.5, P<0.022) variation between sexes of *E. quadripustulatus*. For example, the mean (±SE) developmental period from egg to pupa was 107.5±8.35 days in male *E. quadripustulatus* and 97.83±3.8 days in female. No interaction ($F^6{}_{70}$=0.76, P>0.6) was observed between developmental stage and sex.

Prepupae

Female prepupae developed relatively faster (2.5±0.56 days) than male (3.5±0.29 days). Overall, all the prepupae developed in a shorter period than other immature stages (Table II).

Table II.- Mean developmental period (days) of *Exochomus quadripustulatus* with *Lepidosaphes ulmi* as prey under ambient conditions in field insectary.

Stage	Male		Female	
	Range	Mean±SE	Range	Mean±SE
Egg	33-48	37.25±3.61	33-35	33.67 ± 0.42
Larva				
1st instar	14-16	14.75±0.48	14-16	14.83±0.31
2nd instar	6-10	7.50±0.96	6-7	6.50±0.22
3rd instar	10-13	11.75±0.75	9-12	10.50±0.43
4th instar	17-24	21.00±1.78	15-25	18.33±1.43
Pre-Pupa	3-4	3.50±0.29	1-4	2.50±0.56
Pupa	11-13	11.75±0.48	10-13	11.50±0.43
Total duration from egg to pupa	94-128	107.5±8.35	88-112	97.83±3.8

Pupae

There was no significant (t_7= 0.39, P>0.71) difference in the developmental period of male (11.75±0.48) and female (11.50±0.43) pupae when a two sample t-test was applied.

Growth of immature stages

Larvae

The mean head capsule widths and body lengths of all larval instars showed highly significant (Y=0.101X+0.225, r=0.99, P<0.001) relationship between all the four larval instars. The mean weight of all larval instars increased geometrically (Fig.2).

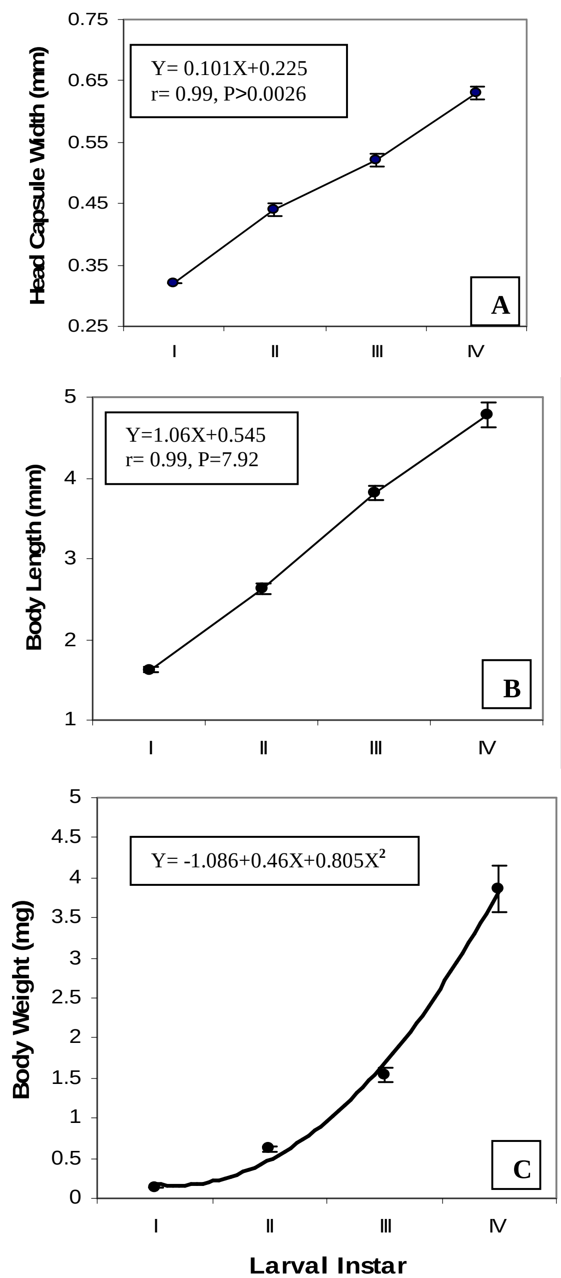

Fig. 2. Relationship between larval instar and measurements made on head capsule, body length, and weight of *Exochomus quadripustulatus*, **(**A) width of head capsule, (B) body length of each larval insatr with fitted regression line, and (C) body weight plotted against each larval instar fitted with second degree polynomial regression equation.

Pupae

Pupal size was not quite significantly related to adult size (Fig. 3) but was just related (P<0.05) to adult weight. Two sample t-tests for insectary reared pupae showed no significant difference (t_7=2.17, P>0.06) between the male (8.58±0.34 mm) and female (9.95±0.53 mm). On the contrary, a highly significant (t_{173}=5.78, P<0.001) difference was observed between field collected male (*n*=106, 13.76±0.12 mm) and female (*n*=79, 14.76±0.13 mm) pupal size. In general, the insectary reared pupae were smaller than field collected ones. The insectary reared pupal period (≥11 days) of *E. quadripustulatus* was also approximately similar to those collected from the field.

Variation in insectary reared adults

Figure 4 shows a significant linear relationship between body length and fresh weight of both males and females (Y=3.52X-8.82, r=0.93, P<0.001). Females were heavier than males i.e; 5.67±0.41 mg and 3.90±33 mg, respectively (t_7=3.35, P<0.012). The body size (length x breadth) of females (11.76±0.49 mm) was significantly (t_7=4.84, P<0.005) greater than that of male (8.53±0.46 mm). The insectary reared individuals were both lighter in weight and smaller compared to the field studied beetles.

Adult longevity

More females than males survived for long periods when fed upon *L. ulmi* under insectary conditions. For example, of ten insectary reared *E. quadripustulatus*, two male beetles survived for 53 and 114 days while the remaining two died after 8-9 days either accidently or by natural death. Similarly, of six female beetles, three survived for 299-305 days and the remaining three beetles died after 12-28 days by natural death. Despite the fact that the insectary reared adult beetles at eclosion were lighter in weight, they survived succesfully. The longevities of male and female adult *E. quadripustulatus* determined in the present insectary study were approximately similar to those beetles which were kept under the same conditions in a separate longevity experiment.

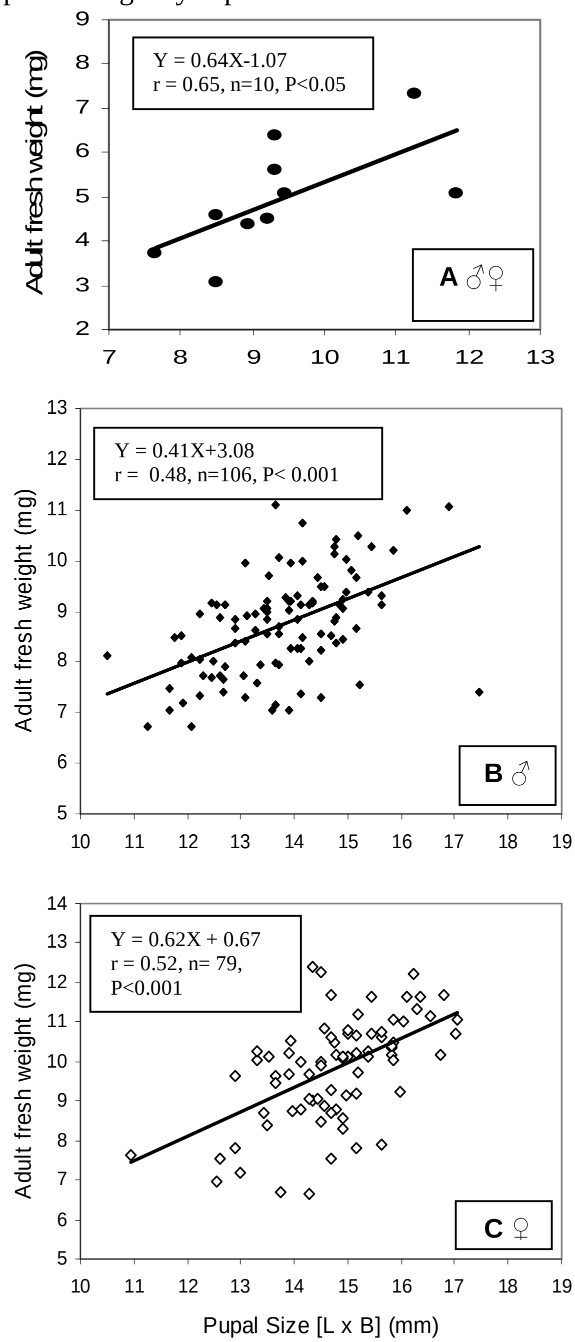

Fig. 3. Relationship between adult fresh weight and pupal size of insectary reared and field collected pupae of *Exochomus quadripustulatus,* (A) insectary reared under ambient conditions male & female adult weight (mg) plotted against pupal size; (B) male adult weight plotted against field collected pupae size; (C) female adult weight plotted against field collected pupae size.

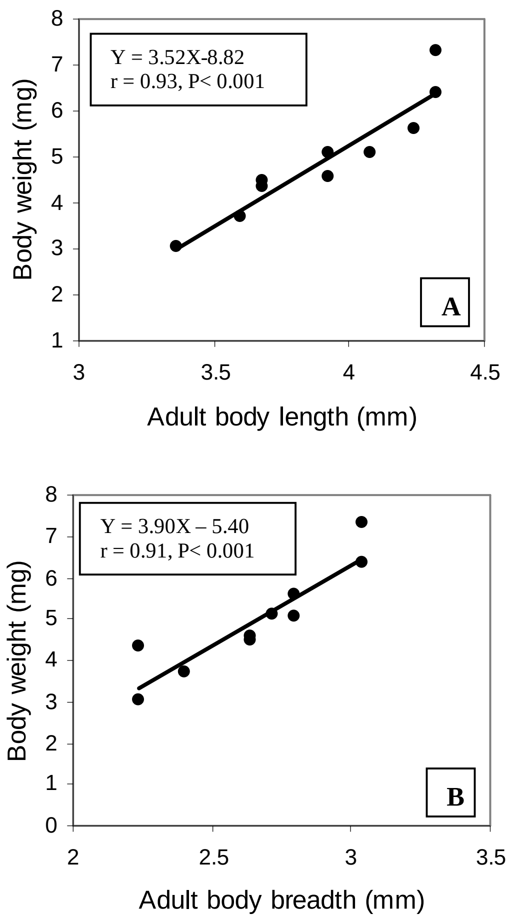

Fig. 4. Relationship between insectary reared under ambient conditions male & female adult body weight (mg) and length/breadth (mm) of *Exochomus quadripustulatus* (A) Body weight plotted against adult body length; (B) Body weight plotted against adult breadth.

DISCUSSION

The egg-laying behaviour utilising the layers of tissue paper as an oviposition site suggests that female normally lays her eggs inside protected places such as the crevices of loose bark in the field (Smith and Coppel, 1957). Although the present findings reflect an incomplete record of fecundity, the rate of oviposition per day per female was slightly higher (2.02 eggs/day/female) than those recorded by Radwan and Lövei (1983) who introduced the coccid *Parthenolecanium corni* (Bouché) to adult *E. quadripustulatus*. The authors further observed different rate of egg laying when they offered two different aphid species. For example, females laid 2.6 and 4.3 eggs/day when *Acyrthosiphon pisum* and *Dysaphis plantaginea* were provided, respectively. A considerable variation in the rate of oviposition was also reported by Uygun (1978) who observed that females lay 1 to 32 eggs/day when fed upon citrus scale *Planococcus citri*. Possible explanations of this variable behaviour of egg-laying, could be either due to differences in food species such that in the field the beetle is known to feed on more than one prey *i.e.,* aphid and coccid, or environmental factors particularly the temperature. For instance, Geyer (1947) found that high temperature (30°C) compared to low (24°C) persuade the females of *Exochomus flavipes* Thunb to lay large number of eggs/day. Tondeur *et al*. (1993) related variable oviposition in *E. quadripustulatus* to changes in the coccid *Eupulvinaria hydrangeae* life stages as newly produced ovisacs increased egg-laying of the predator. Findings concerning late development of eggs are in accordance to those studied by Wilson (1938) under ambient conditions of England. Under the field conditions, the peak hatching time showed a good synchronisation between the predator (emergence of first instar larva) and prey (appearance of crawlers)_(unpublished data).

The data suggest that the growth in various stages of *E. quadripustulatus* are as described by Hodek (1973) for coccinellids in general and Uygun (1978) for *E. quadripustulatus*, in particular. The increase in head width of larvae of *E. quadripustulatus* in successive instars followed a regular geometric relationship as cited by Rahoo (1985). The weight of all the four larval instars increased as the successive instar stage appeared which suggest that early instars consumed less food compared to the last. This may be due to the early instars being less mobile feeding nearby the host compared to third and fourth instars which are more active and capable of wandering in search of food. Radwn and Lövei (1983) also recorded heavier larvae in later instars than earlier but the difference in weight between present studied larvae and theirs is obvious. This variation could certainly be due either to the difference in weighing procedure or to the type of food provided. For instance, in this study the weight of the larva was recorded soon after ecdysis whilst Radwan and Lövei (1983) weighed them after every 2-3 days interval till prepupal stage. Similarly, in this study, the development of all the larval instars was accomplished on different stages of *L. ulmi* whereas these authors studied the larval development not only on different prey species but also provided a third larval instars of various aphid species. Generally the speed of development of coccinellids depends upon the ambient temperature, as the favourable range of temperature accelerates the rate of development in all the stages (Hodek, 1973). In the present work, the development of the four larval stages of *E. quadripustulatus* under fluctuating conditions, took longer than other stages. These findings agree with those recorded by Uygun (1978) who observed that larval development takes ≥7 weeks at 15-25°C. Although the author concluded that the larval development is temperature dependent, however, Radwan and Lövei (1983) recorded 18-20 days at a constant temperature 23°C.

During the present investigations, the shortest period of development was recorded at the prepupal stage. Wilson (1938) recorded two days prepupal stage in England which seems similar to the present findings.

Blanckenhorn (1994) showed that higher food levels increased the reproductive output of females but not their longevity or oviposition period in *Aquarius remigis* (Heteroptera: Gerridae). The variation in size could be related to the amount of food consumed during the larval stage. Thus heavier larvae pupated into larger adults. The slight linear relationship between pupal size and adult fresh weight suggests that heavier adults may emerge from the larger sizes of pupae. The differences between newly emerged male and female adult body weight could be that females eat more during summer than males in order to lay eggs in the following season. The size and weight differences was also recorded by Henderson and Albrecht (1988*b*) in field-collected samples of *E. quadripustulatus*. Smith (1966) concluded that weight and size of coccinellid adults varied with species, sex, feeding, and females are more variable than males in body size and weight. However, weight and size relationships could be used as a good indicator in determining the fecundity, fertility and longevity within subject species. Measuring weight of adults is a more efficient and less time consuming procedure than size. Hattingh and Samways (1994) while studying the coccinellid *Chilocorus* spp. also considered that adult weight proportional to the size is widely accepted in determining the fecundity of individuals within species. The survival of some males and females for a period of ≥4 and ≥10 months respectively, indicate that predatory beetles are capable of surviving for a long time when preying upon *L. ulmi*.

Although the effectiveness of *E. quadripustulatus* against scale insects particularly *L. ulmi* in the field and laboratory is not known, this study provides valuable information in understanding the development of this coccinellid beetle on mussel scale, which has not yet been investigated. The present data indicate that this species is probably capable of maintaining its development on scale insects alone when the alternate prey such as aphids are not available in the field.

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