

## PERSPECTIVE

# Using viable eggs to accurately determine the demographic and predation potential of *Harmonia dimidiata* (Coleoptera: Coccinellidae)

D.-F. Mou<sup>1</sup>, C.-C. Lee<sup>1</sup>, C. L. Smith<sup>2</sup> & H. Chi<sup>1</sup>

<sup>1</sup> Laboratory of Theoretical and Applied Ecology, Department of Entomology, National Chung Hsing University, Taichung, Taiwan, China

<sup>2</sup> Georgia Museum of Natural History, University of Georgia Athens, GA, USA

## Keywords

biological control, egg hatch rate, population projection, predation rate, two-sex life table

## Correspondence

Hsin Chi (corresponding author), Laboratory of Theoretical and Applied Ecology, Department of Entomology, National Chung Hsing University, PO Box 17-25, Taichung 402, Taiwan, China.  
E-mail: hsinchi@dragon.nchu.edu.tw

Received: June 15, 2014; accepted: December 4, 2014.

doi: 10.1111/jen.12202

## Abstract

Life table parameters and predation rate of the coccinellid predator *Harmonia dimidiata* F. fed on *Aphis gossypii* Glover were determined at 25°C using the age-stage, two-sex life table. When the total number of eggs were included in our calculations, the intrinsic rate of increase ( $r$ ), finite rate ( $\lambda$ ), net reproductive rate ( $R_0$ ) and mean generation time ( $T$ ) for *H. dimidiata* were 0.1354/day, 1.1450/day, 280.8 offspring and 41.6 day, respectively. These values were significantly different, however, only when viable (=hatchable) *H. dimidiata* eggs were counted (0.0909/day, 1.0952/day, 67.6 offspring and 46.3 day, respectively). The values obtained using all eggs did not realistically reflect the effect of variable hatch rate and true population parameters of *H. dimidiata*. We therefore excluded unhatched eggs from our data and demonstrated mathematically that in future demographic studies, it should be a standard procedure to exclude all unhatched eggs when analysing hatch rates that vary with maternal age. A mathematical proof was derived in this study to substantiate this. To observe and quantify variations that occur in the predation rate due to the age and stage of the predator, the daily number of *A. gossypii* consumed by individual *H. dimidiata* was analysed using the age-stage, two-sex life table. The net predation rate of *H. dimidiata* on *A. gossypii* was 14 804 aphids. The transformation rate  $Q_p$  showed that the predator needs to consume an average of 219.1 aphids to produce one viable egg. The finite predation rate of *H. dimidiata* was 125.7 when only hatchable eggs were included in the analysis. Because the age-stage, two-sex life table takes both of the sexes and the variable predation rate occurring among stages into consideration, it becomes possible to use the population projection to quantify and time biological control procedures.

## Introduction

Biological control is potentially one of the most important strategies available in pest control. This is especially true with the increasing popularity of organic farming. To formulate a successful biological control programme, it is essential to construct and analyse life tables of both predator and prey and to integrate these into the programme in a timely

fashion. Life tables provide the only comprehensive description of the survival, developmental and reproductive rates of a population (Lotka 1907; Lewis 1942; Leslie 1945, 1948; Birch 1948; Messenger 1964; Chi and Liu 1985; Carey 1993) and have frequently been utilized in human demographic studies for many decades.

Chi and Liu (1985) and Chi (1988) developed the age-stage, two-sex life table theory to incorporate the

contributions made by both sexes and to enable an accurate description of the stage differentiation that occurs in insect and mite populations. Since then, predation studies based on the age-stage, two-sex life table have increased (Yu et al. 2005, 2013a,b; Chi and Su 2006; Farhadi et al. 2011). To enable the effective comparison of the predation potential of different predators, Chi et al. (2011) and Yu et al. (2013a) developed the finite predation rate, a composite of the age-stage structure, population growth rate and predation rate.

In life table analysis, the survival rate and fecundity are two major parameters. Although Livdahl and Edgerly (1987) discussed factors affecting the hatch rate of insects, and Fox (1993) noticed that egg hatchability of *Callosobruchus maculatus* (Coleoptera: Bruchidae) decreased with increasing maternal age, few authors have noted the importance of egg hatch rate in data analysis, for example Jha et al. (2012a) noticed females of *Helicoverpa armigera* (Hübner) produced more unhatched eggs at older ages, and Tuan et al. (2013) observed a similar phenomenon in *Spo-doptera litura* (F.). Jha et al. (2012a,b) addressed the importance of excluding unhatched eggs to obtain realistic demographic characteristics and avoid the overestimation of population parameters.

The aphidophagous ladybird beetle, *Harmonia dimidiata* F. (Coleoptera: Coccinellidae), is a common, widely distributed natural enemy of many aphid species (Tao 1990; Chen et al. 2009). Agarwala et al. (2009) reported an asymptotic predation rate of *H. dimidiata*, of 100 *Cervaphis quercus* (Takahashi) per female. Gillani et al. (2007) reported that female *H. dimidiata* survived on average of 81 days when fed on *Brevicoryne brassicae* (L.). Yu et al. (2013a) reported a net predation rate as high as 7492 aphids per individual *H. dimidiata* on *Aphis gossypii* at 25°C. The predation rate of *H. dimidiata* fed on *A. gossypii* also changed with age and stage at three different temperatures (15, 20 and 25°C) (Yu et al. 2013a).

To accurately estimate the population parameters and predation capacity by taking the variable hatch rate into consideration, we compared the life table parameters calculated from data generated using all eggs (both hatched and unhatched) versus data generated using only hatched eggs in this study. Furthermore, to incorporate the predation rate with the life table, we analysed the predation rate raw data of *H. dimidiata* using the age-stage, two-sex life table. Finally, we used computer simulation to project the population growth and change of predation capacity of the predator to facilitate proper release timing of *H. dimidiata*.

## Materials and Methods

### Culturing of *Aphis gossypii* and *Harmonia dimidiata*

The melon aphid, *A. gossypii*, were reared on leaves of the muskmelon, *Cucumis melo* L., variety 'Autumn Favor' (Known-You Seed Co., Ltd., Kaohsiung, Taiwan). Melon aphids were used as the prey for *H. dimidiata* in this study. Six pairs of adult *H. dimidiata* were obtained from a colony maintained at the Taiwan Agricultural Research Institute and originating from Fusing Township, Taoyuan County, Taiwan. Paired *H. dimidiata* were cultured in plastic boxes (11 by 7.5 by 5 cm<sup>3</sup>) supplied with *A. gossypii* fed on a melon leaf and kept in a growth chamber set at 25°C, 80% RH and a photoperiod of 12 : 12 (L : D) h. A rectangular hole (10 by 7 cm<sup>2</sup>) was cut in the top of each plastic box and covered with fine mesh nylon net for ventilation.

### Life table and predation rate study of *Harmonia dimidiata*

83

A total of 84 *H. dimidiata* eggs laid on a melon leaf within a 24-h period were collected and maintained in new plastic boxes for use in the life table and predation rate studies. Each newly hatched first instar ladybird beetle was moved into an individual plastic box (11 by 7.5 by 5 cm<sup>3</sup>) and kept in a growth chamber set at the aforementioned conditions. According to Yang (1994), no significant differences were found in the developmental time and population parameters between *P. japonica* individuals fed entirely on third stage *M. persicae* and those fed on mixed stages of *M. persicae*. Due to the extensive amount of time and effort involved in life table and predation rate studies, we sought to simplify his procedure by providing each *H. dimidiata* larva 550–600 mixed stage aphids on a melon leaf per day. The number of aphids was counted prior to adding to each *H. dimidiata* cage, and the remaining aphids were recounted every subsequent 24-h period to determine the daily predation rate. Each ladybird beetle was provided with a new leaf daily containing 550–600 aphids. Survival and development were also recorded daily until pupation of the beetles. Newly emerged adults were paired and provided a new melon leaf containing 750–850 aphids of mixed stages daily. The number of aphids was counted before and after to determine the daily predation rate, and a new leaf with 750–850 aphids was provided to the paired adults. Because adults were paired in the rearing boxes, it was not practical to quantify any possible predation difference between female and male *H. dimidiata*. Instead, we recorded the total number of aphids

consumed by each pair of adult ladybird beetles. We then calculated the mean number of aphids consumed by each adult ladybird beetle without distinguishing between male and female. If an individual died before its mate, another adult of the same sex was recruited from the mass-rearing colony and paired with the surviving individual. Data relevant to the recruited individual were not included in the data analysis. The survival, development, daily predation rate and fecundity were recorded every 24 h until the death of each individual. The eggs laid by each female *H. dimidiata* at different ages were kept separately in plastic boxes under the aforementioned conditions. The number of hatched eggs was recorded daily.

### Life table analysis

Raw data on the development, survival and daily fecundity of *H. dimidiata* were analysed according to the age-stage, two-sex life table (Chi and Liu 1985; Chi 1988). The computer program, TWOSEX-MSChart (Chi 2013a), was used to facilitate the data analysis. Using the methods described by Chi and Liu (1985), the age-stage-specific survival rate ( $s_{xj}$ , where  $x$  is age and  $j$  is stage); the age-specific survival rate ( $l_x$ ); the age-stage-specific fecundity ( $f_{xj}$ ); the age-specific fecundity ( $m_x$ ); and the population parameters (the intrinsic rate of increase,  $r$ ; the finite rate of increase,  $\lambda$ ; the net reproductive rate,  $R_0$ ; and the mean generation time,  $T$ ) were calculated. The  $s_{xj}$  represents the probability that a newborn egg will survive to age  $x$  and stage  $j$ . The  $l_x$  represents the probability that a newborn egg will survive to age  $x$  and was calculated using the following equation:

$$l_x = \sum_{j=1}^{\delta} s_{xj}, \quad (1)$$

where  $\delta$  is the last stage of the study cohort. The  $m_x$  was calculated using the following equation:

$$m_x = \frac{\sum_{j=1}^{\delta} s_{xj} f_{xj}}{\sum_{j=1}^{\delta} s_{xj}}. \quad (2)$$

The net reproductive rate ( $R_0$ ) is the total number of offspring produced by an average individual during its lifetime and was calculated as follows:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x. \quad (3)$$

The intrinsic rate of increase ( $r$ ) was calculated using the iterative bisection method and the

Euler–Lotka equation with age indexed from 0 (Goodman 1982):

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1. \quad (4)$$

The finite rate ( $\lambda$ ) and mean generation time ( $T$ ) were calculated as follows:

$$\lambda = e^r. \quad (5)$$

$$T = \frac{\ln R_0}{r}. \quad (6)$$

We initially analysed the daily raw data for development and survival of all 84 eggs used at the beginning and the daily total eggs produced by the 23 females that emerged from these 84 eggs to calculate the total age-specific fecundity ( $E_x$ ) and the population parameters. We then used only those 54 eggs that hatched from the original 84 eggs and the hatched eggs of the daily fecundity of the 23 females that emerged from those 54 hatched eggs to calculate the age-specific hatched eggs ( $H_x$ ) and the population parameters. The hatch rate ( $h_x$ ) of *H. dimidiata* was obtained by dividing the number of hatched eggs ( $H_x$ ) with the total number of eggs ( $E_x$ ) laid by females at age  $x$ . The variances and standard errors of population parameters of *H. dimidiata* were estimated using bootstrap techniques (Efron and Tibshirani 1993) with 40 000 bootstrap samples for more stable and precise estimates.

### Predation rate analysis

To correlate the predation rate with variations in age and stage, daily predation rates were also analysed according to the two-sex life table using CONSUME-MSChart (Chi 2013b). Following the method of Chi and Yang (2003), the age-stage-specific predation rate ( $c_{xj}$ , where  $x$  is age and  $j$  is stage) was calculated. This is the mean number of *A. gossypii* consumed by an individual *H. dimidiata* at age  $x$  and stage  $j$ . The age-specific predation rate ( $k_x$ ) is the mean number of aphids consumed by *H. dimidiata* at age  $x$ , and it was calculated as follows:

$$k_x = \frac{\sum_{j=1}^{\delta} s_{xj} c_{xj}}{\sum_{j=1}^{\delta} s_{xj}}. \quad (7)$$

Taking the survival rate into consideration, the age-specific net predation rate ( $q_x$ ) was calculated using the following equation:

$$q_x = k_x l_x. \quad (8)$$

The cumulative predation rate ( $C_x$ ) represents the total number of prey consumed by an average predator from birth up to age  $x$  and is calculated as follows:

$$C_x = \sum_{i=0}^x l_i k_i. \quad (9)$$

The net predation rate ( $C_0$ ) is the number of prey consumed by an average individual predator during its life span and is calculated as follows:

$$C_0 = \sum_{x=0}^{\infty} l_x k_x. \quad (10)$$

The ratio of the net predation rate to the net reproductive rate yields the transformation rate  $Q_p$ ; this is the number of prey needed for a predator to produce a single offspring and is calculated as follows:

$$Q_p = \frac{C_0}{R_0}. \quad (11)$$

To effectively compare predation capacity based on both population growth rate and predation rate, Chi et al. (2011) and Yu et al. (2013a) defined the stable predation rate ( $\psi$ ) as the total predation capacity of a population whose size is unity (meaning the total population size is one) and at stable age-stage distribution (SASD). The  $\psi$  and finite predation rate ( $\omega$ ) are calculated according to the following equation:

$$\psi = \sum_{x=0}^{\infty} \sum_{j=1}^{\delta} a_{xj} c_{xj} \quad (12)$$

$$\omega = \lambda \psi, \quad (13)$$

where  $a_{xj}$  is the proportion of individuals belonging to age  $x$  and stage  $j$  in a SASD. The variances and standard errors of  $C_0$ ,  $Q_p$ ,  $\psi$  and  $\omega$  were estimated using bootstrap techniques (Efron and Tibshirani 1993) with the same 40 000 bootstrap samples used in the life table analysis. For the standard error of  $Q_p$ , the same bootstrap resampling sequences for  $R_0$  were used in the calculation of  $C_0$ . The bootstrap subroutine is included in the TWOSEX-MSChart and CONSUME-MSChart (Chi 2013b) program.

#### Statistical analysis

The differences between population parameters ( $r$ ,  $\lambda$ ,  $R_0$ ,  $T$ ,  $F$ , APOP and TPOP) and predation parameters

( $C_0$ ,  $Q_p$ ,  $\psi$  and  $\omega$ ) calculated using all eggs and hatched eggs were compared using a  $t$ -test at 5% significant level (Efron and Tibshirani 1993). To compare the difference in predation rates among stages, we used the paired bootstrap test based on percentile from results of 40 000 bootstrap samples (Efron and Tibshirani 1993; Brandstätter 1999). Paired bootstrap test was used to compare the difference in predation rates among stages.

#### Computer simulation

Based on the two-sex life table and the daily predation rate obtained in this study, the population growth and predation capacity of *H. dimidiata* was projected using Timing-MSChart (Chi 1990, 2013c). Predation capacity at time  $t$  was calculated as follows:

$$p(t) = \sum_{j=1}^{\delta} \left( c_j \cdot \sum_{x=0}^{\infty} n_{xj}(t) \right), \quad (14)$$

where  $n_{xj}(t)$  is the number of individuals at age  $x$  and stage  $j$  at time  $t$ , and  $c_j$  is the mean daily predation rate of individuals in stage  $j$ . To take the survival rate and developmental duration into account, the  $c_j$  is calculated as

$$c_j = \frac{\sum_{x=0}^{\infty} s_{xj} c_{xj}}{\sum_{x=0}^{\infty} s_{xj}}. \quad (15)$$

In the population projection of a single release, 10 hatchable eggs (aged 3 days) were used as the initial population. In the population projection of a double release, 10 hatchable eggs (aged 3 days) were used as the initial population and an additional 10 hatchable eggs (aged 3 days) were released on the 10th day.

## Results

#### Life table of *Harmonia dimidiata*

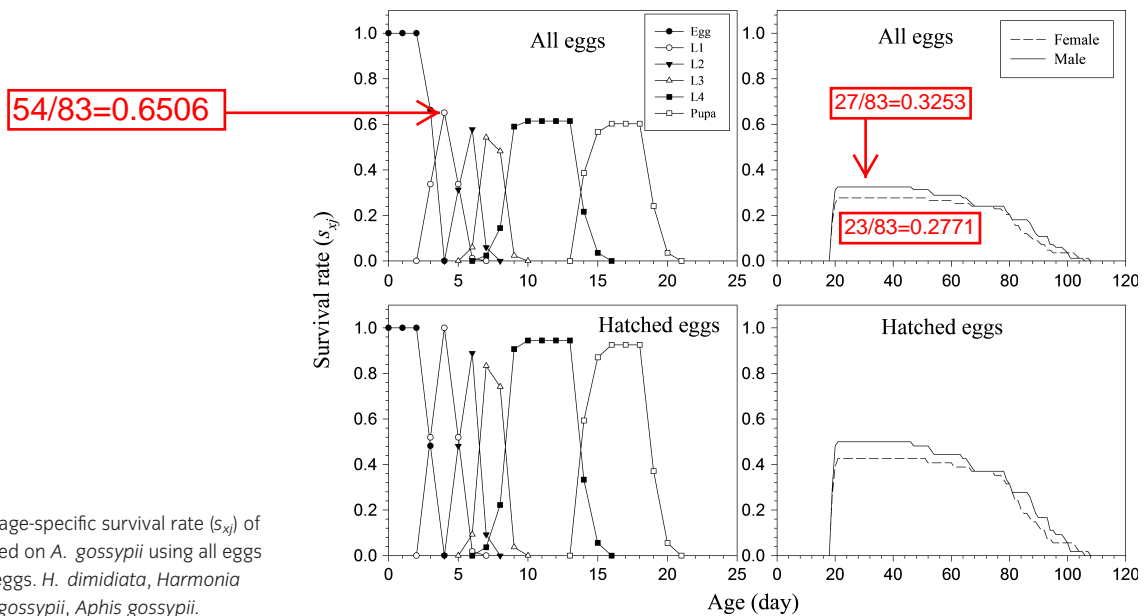
Of the 84 eggs of *H. dimidiata* used at the beginning of the life table study, 54 hatched successfully. The mean developmental times of each stage and the adult longevities are listed in table 1. The mean developmental time of the egg stage was 3.48 days, and the female and male adult longevities were 65 day and 63.33 days, respectively (table 1). Figure 1 illustrates the  $s_{xj}$  of *H. dimidiata*. Because of the variable developmental rate among individuals, there are overlaps between stages. When all 84 eggs were included in our analysis, the  $s_{xj}$  values of *H. dimidiata* are lower due to the low hatchability at the egg stage. When only the hatched eggs were used, that is the mortality

**Table 1** Means and standard errors of developmental time (day), adult longevity (day), stage-specific predation rate (preys/predator) and daily mean predation rate ( $c_j$ ) of *H. dimidiata* fed on *A. gossypii*

Stage	<i>n</i>	Developmental time (day)*	Predation rate (preys/predator)*	Daily mean predation rate ( $c_j$ )*
Egg	54	3.48 ± 0.07	–	–
First instar	54	2.06 ± 0.04	86.4 ± 5.8 a	42.05 ± 1.87 a
Second instar	52	1.44 ± 0.07	225.2 ± 15.4 b	152.76 ± 5.06 b
Third instar	51	1.76 ± 0.06	429.8 ± 18.5 c	244.53 ± 6.06 d
Fourth instar	50	5.66 ± 0.09	1190.0 ± 37.7 d	209.69 ± 5.40 c
Pupa	50	5.04 ± 0.03	–	–
Female adult	23	65.00 ± 2.73	–	–
Male adult	27	63.33 ± 3.07	–	–
Adult (both sexes)	50	64.10 ± 2.06	13 996.3 ± 441.3 e	218.35 ± 1.05 c

*H. dimidiata*, *Harmonia dimidiata*; *A. gossypii*, *Aphis gossypii*.

\*All standard errors were estimated using bootstrap technique (Efron and Tibshirani 1993). Predation rates followed by different letters are significantly different based on paired bootstrap test (Efron and Tibshirani 1993; Brandstätter 1999).



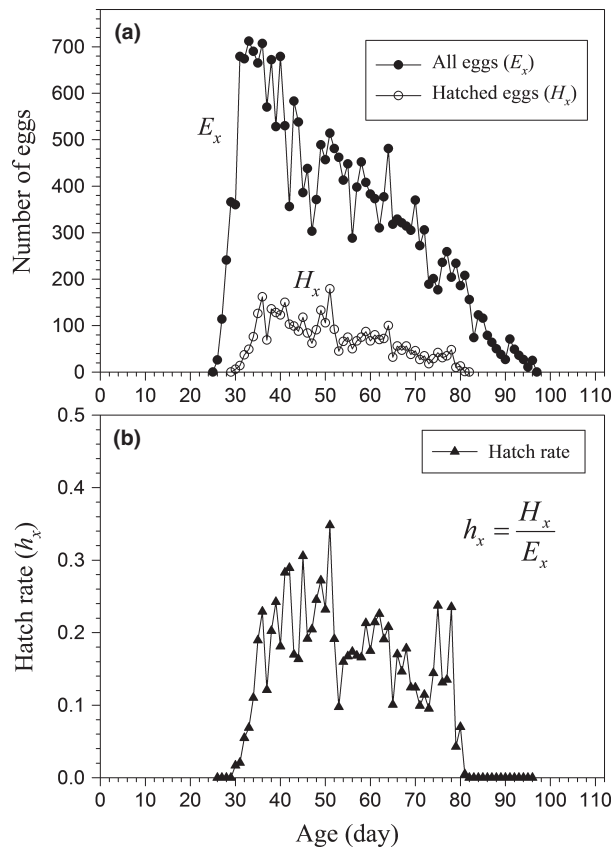
**Fig. 1** Age-stage-specific survival rate ( $s_{xj}$ ) of *H. dimidiata* fed on *A. gossypii* using all eggs and hatched eggs. *H. dimidiata*, *Harmonia dimidiata*; *A. gossypii*, *Aphis gossypii*.

in the egg stage was excluded, the curves of  $s_{xj}$  were higher (fig. 1).

The total number of eggs ( $E_x$ ) produced by the *H. dimidiata* cohort and the total number of hatched eggs ( $H_x$ ) are displayed in fig. 2a. The mean hatch rate was 0.1528, and the maximal hatch rate was 0.3482 at age 51 days (counting from birth), that is 24 days after the initiation of egg production (fig. 2b). These figures clearly showed that not only the oviposition but also the hatch rate varied with the maternal age. For example, all eggs produced during the first 4 days (age 26–29 days) were inviable; similarly, all eggs laid during the last 15 days of reproduction (age 82–96 days) were also inviable. Figure 3 compares the  $l_x$ ,  $m_x$  and  $f_{x7}$  of *H. dimidiata* estimated using all eggs and

hatched eggs. Because there were no mortality between age 15 and 47 days, the curves were flat during that period (fig. 3a). The difference between  $l_x$  with all eggs and with only hatched eggs is mainly due to the mortality in the egg stage as shown in the curve of  $s_{xj}$  (fig. 1). The  $f_{x7}$  is the mean number of eggs produced by a female adult at age  $x$ . The  $f_{x7}$  curve calculated using hatched eggs represented the effective mean fecundity (i.e. viable eggs) produced by a female at age  $x$ .

The population parameters of *H. dimidiata* that were estimated using the bootstrap technique are presented in table 2. When all eggs were included in the life table analysis,  $R_0$  was as high as 280.8 offspring per individual. However, it decreased to 67.6 when only



**Fig. 2** (a) Number of eggs ( $E_x$ ) and hatched eggs ( $H_x$ ) laid by *H. dimidiata* at age  $x$ . (b) Age-specific hatch rate ( $h_x = H_x/E_x$ ) of *H. dimidiata*. *H. dimidiata*, *Harmonia dimidiata*.

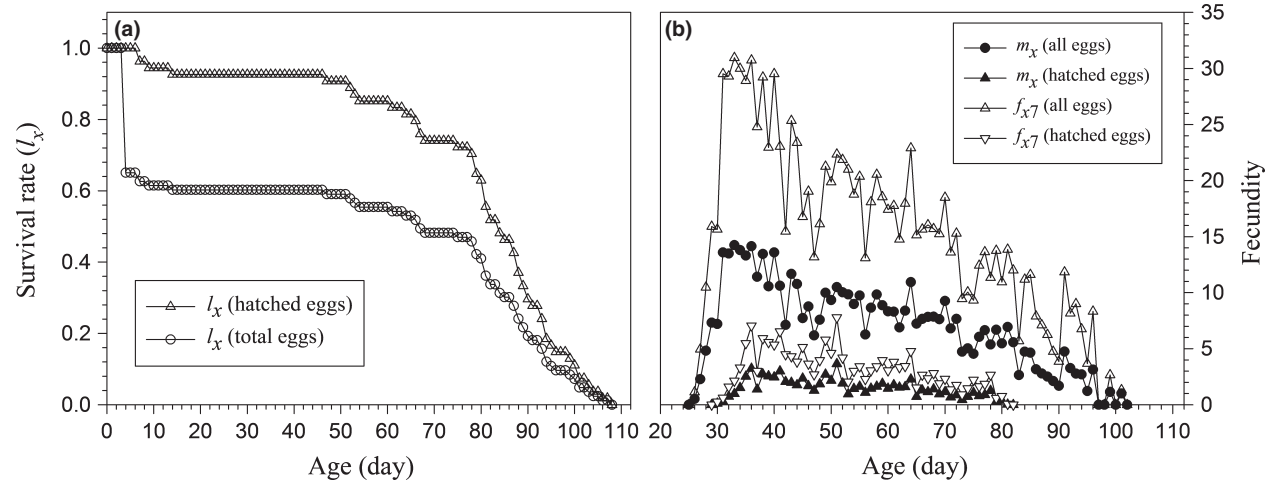
hatched eggs were included in the analysis. The intrinsic rate of increase ( $r = 0.0909/\text{day}$ ) and finite rate ( $\lambda = 1.0952/\text{day}$ ) based on hatched eggs were

also significantly lower than those based on all eggs ( $r = 0.1354/\text{day}$  and  $\lambda = 1.1450/\text{day}$ ).

**Predation rate of *Harmonia dimidiata***

In general statistics, the standard error (SE) of predation rate is estimated using data of all individuals in a stage (e.g. fig. 4a,  $n = 50$  for predation rate of the fourth instar); while in the bootstrap method, the SE is estimated using means of all resampling (fig. 4b,  $m = 40\ 000$ ). As shown in fig. 4b–d, bootstrap generated a normal frequency distribution which is essential for the ensuing analysis and comparison. The bootstrap results were also consistent with the definition of the central limit theorem ‘As sample size increases, the means of samples drawn from a population of any distribution will approach the normal distribution’. The subsequent paired comparison based on confidential intervals of differences showed there were significant differences among stages (table 2).

It was determined that the hatch rate of *H. dimidiata* eggs changed with the maternal age. To minimize this variability, we estimated the predation parameters of *H. dimidiata* using only the number of hatched eggs. There were two obvious gaps in the curve of  $c_{xj}$ ,  $k_x$  and  $q_x$  (fig. 5). These gaps are due to the non-predatory egg and pupal stages (including pre-pupal stage). The daily mean predation rate increased from the first to the fourth instar, with the maximum predation rate observed during the fourth instar. The predation rate decreased at the end of the fourth instar when larvae entered the pre-pupal stage. Because the predation rate of the adult stage was



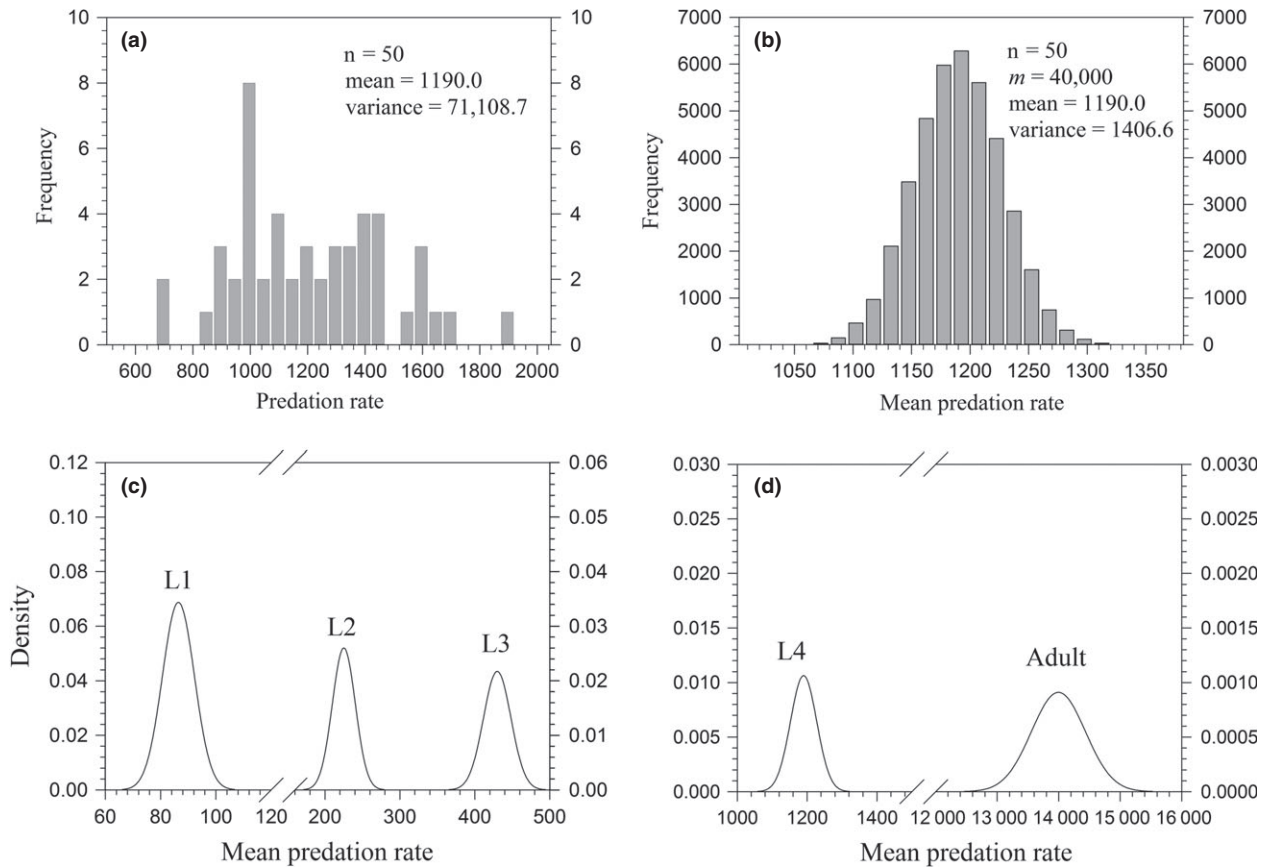
**Fig. 3** (a) Age-specific survival rate ( $l_x$ ), (b) age-specific fecundity ( $m_x$ ) and age-stage-specific fecundity ( $f_{x7}$ ) of *H. dimidiata* using all eggs and hatched eggs. *H. dimidiata*, *Harmonia dimidiata*.

**Table 2** Means and standard errors of population parameters and predation rates of *H. dimidiata* fed on *A. gossypii* using all eggs and hatched eggs (bootstrap technique with 40 000 resamplings, d.f. = 135 for all parameters)

Parameters	All eggs	Hatched eggs	<i>t</i>	P
<i>r</i> (/day)	0.1354 ± 0.0056	0.0909 ± 0.0061	43.0	<0.0001
$\lambda$ (/day)	1.1450 ± 0.0064	1.0952 ± 0.0066	43.3	<0.0001
<i>R</i> <sub>0</sub> (offspring/individual)	280.8 ± 57.5	67.6 ± 17.8	31.4	<0.0001
<i>T</i> (day)	41.6 ± 0.8	46.3 ± 1.0	28.1	<0.0001
<i>F</i> (eggs/female)	1013.4 ± 105.2	159.0 ± 33.7	68.4	<0.0001
APOP (day)	8.5 ± 0.3	16.1 ± 1.6	34.5	<0.0001
TPOP (day)	28.0 ± 0.3	35.7 ± 1.6	34.7	<0.0001
<i>C</i> <sub>0</sub> (preys/predator)	9631 ± 895	14 804 ± 678	38.4	<0.0001
<i>Q</i> <sub>p</sub> (preys/offspring of predator)	34.3 ± 6.9	219.1 ± 68.9	21.2	<0.0001
$\psi$ (Stable predation rate, preys)	75.9 ± 3.6	114.8 ± 2.1	79.6	<0.0001
$\omega$ (Finite predation rate, preys)	86.9 ± 4.4	125.7 ± 2.4	67.5	<0.0001

*H. dimidiata*, *Harmonia dimidiata*; *A. gossypii*, *Aphis gossypii*.

There are significant differences between parameters calculated using all eggs and hatched eggs according to *t*-test (*P* < 0.0001) at 5% significant level based on bootstrap technique (Efron and Tibshirani 1993).



**Fig. 4** (a) Observed frequency of predation rate of fourth instar *H. dimidiata* (50 individuals). (b) Frequency distribution of mean predation rate estimated using bootstrap (*m* = 40 000). (c) and (d) Normal density curves of mean predation rates of different *H. dimidiata* stages estimated using bootstrap technique. *H. dimidiata*, *Harmonia dimidiata*.

calculated using paired adults (male and female), it was not feasible to extrapolate from these data any possible differences that may exist in the predation

rate between the two sexes. The *c*<sub>*xj*</sub> of *H. dimidiata* adults shows the average predation rate of an individual adult at age *x* without distinguishing the sex

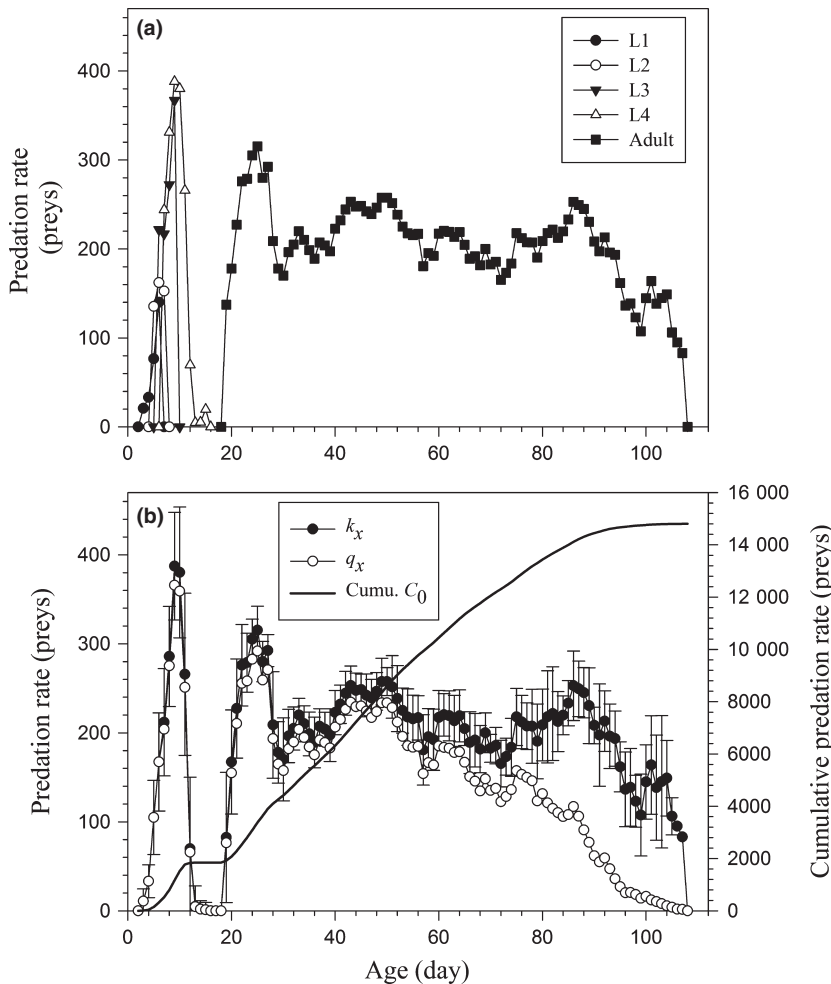
of the individual. The total numbers of aphids consumed by *H. dimidiata* during each developmental stage are listed in table 1. The highest  $c_{xj}$ , 388.1 prey aphids, was observed during the fourth instar at age 9 days (fig. 5a), while the highest total stage predation rate of 1190 aphids was also observed during the fourth instar (table 1). Adult *H. dimidiata* individuals consumed as many as 13 996.3 melon aphids. Due to the shorter developmental time, the highest stage-specific daily mean predation rate ( $c_j$ ) was observed in the third instar, that is 244.53 prey aphids (table 1).

The net predation rate ( $C_0$ ) was 9631 aphids per ladybird beetle when estimated using all eggs. A higher  $C_0$  of 14 804 aphids was obtained for *H. dimidiata* when only hatched eggs were included in the analysis (table 2). The  $Q_p$  shows that when all eggs were included, *H. dimidiata* required an average of 34.3 aphids to produce a single predator egg. When only hatched eggs were included in the analysis, this

value increased to an average of 219.1 aphids needed to produce one hatchable egg (table 2).

Computer simulation

By combining the life table data and stage-specific daily consumption rate ( $c_j$ ) (table 1), the age-stage structure and the predation capacity of the simulated population of *H. dimidiata* are shown in fig. 6. Because the projection, based on the age-stage, two-sex life table, can reveal the change of stage structure during the population growth, it can reveal the change of predation capacity due to stage differentiation. For example, in the single release simulation, the predation capacity  $p(t)$  dropped at  $t = 11$ , when some of the larvae entered the pupal stage, resulting in a significant gap of predation capacity (on day 13–15) (fig. 6a). After several trials, it was found that a second release of 10 3-day-old eggs on the 10th day would negate the predation capacity gap (fig. 6b).



**Fig. 5** (a) Age-stage-specific predation rate ( $c_{xj}$ ) of *H. dimidiata*. (b) Age-specific predation rate ( $k_x$ ) with error bar (SD), age-specific net predation rate ( $q_x$ ) and cumulative net predation rate of *H. dimidiata* calculated using hatched eggs. *H. dimidiata*, *Harmonia dimidiata*.



**Discussion**

*Life table of Harmonia dimidiata*

The mean longevities of female and male adults were 65.0 and 63.33 days, respectively. These were similar to the values reported by Yu et al. (2013a) using identical temperature and prey, demonstrating the stability of *H. dimidiata* growth performance at 25°C. The percentage of egg hatchability of 64.3% found for the parent cohort (84 eggs) was lower than the 96% reported by Gillani et al. (2007). Although the total eggs laid per female was as high as 1013.4, only 159 eggs per female hatched; therefore, when all eggs were included, a lower hatch rate of 15.3% was obtained in this study. Although most insect pests are *r*-selected with high fecundity, egg hatch rates are affected by various factors (Davidson 1944; Livdahl and Ederly 1987). Perry and Roitberg (2005) reported that ladybird mothers produced trophic eggs to mitigate offspring starvation risk. This might be one reason for the low hatch rate of *H. dimidiata*. Because the hatch rate did vary with the maternal age of *H. dimidiata* (fig. 2), the hatch rate of 0.643 calculated from the parent cohort (84 eggs) represented only that egg batch randomly sampled at the beginning of this life table study. Thus, any egg batch sampled for life table study will differentially influence the population parameters by its hatch rate. This demonstrates the importance of using only the hatched eggs of the parent cohort when collecting survival and development data ( $s_{xj}$  and  $l_x$ ) of the cohort; furthermore, when calculating fecundity ( $f_{xj}$  and  $m_x$ ), it is equally important to use only the hatched eggs of the daily oviposited eggs. In this way, the population parameters will not be affected by the hatch rate of the parent cohort sampled for life table study. Because the hatch rate of *H. dimidiata* eggs varied with maternal age (fig. 3), the life table statistics calculated using only hatched eggs represent the true demographic characteristics of the population (table 2).

To mathematically demonstrate the effect of hatch rate on a population, we derived the following proof based on age-specific survival  $l_x$  and fecundity  $m_x$ . Assuming that  $N$  eggs are collected for the life table study at  $t = 0$  and only  $N_e$  eggs hatched, then the survival rate ( $l_x$ ) must be as follows:

$$l_0 = \frac{N_0}{N} = 1, l_1 = \frac{N_1}{N}, \dots, l_x = \frac{N_x}{N}, \quad (16)$$

where  $N_x$  is the number of survived individuals at age  $x$  and  $N_0 = N$  at  $t = 0$ . The age-specific fecundity is then:

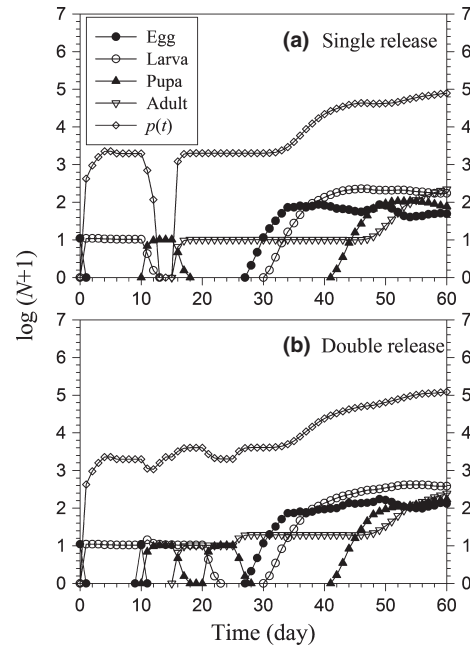
65.06%

83

trophic

0.6506

83



**Fig. 6** Population projection: (a) Single release of 10 eggs of *H. dimidiata* aged 3 days at  $t = 0$ . (b) Double releases of 10 eggs of *H. dimidiata* aged 3 days at  $t = 0$  and  $t = 10$ ,  $p(t)$ , predation capacity. *H. dimidiata*, *Harmonia dimidiata*.

$$m_0 = \frac{0}{N}, m_1 = \frac{0}{N_1}, \dots, m_x = \frac{E_x}{N_x}, \quad (17)$$

where  $E_x$  is the total number of eggs produced by the surviving individuals at age  $x$ , that is  $N_x$ . The net reproductive rate is then:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x = \sum_{x=0}^{\infty} \frac{N_x}{N} \times \frac{E_x}{N_x} = \sum_{x=0}^{\infty} \frac{E_x}{N}. \quad (18)$$

If only hatched eggs are used in life table analysis, then the  $l_x$  sequence will be as follows:

$$l_0 = \frac{N_e}{N_e} = 1, l_1 = \frac{N_{e,1}}{N_e}, \dots, l_x = \frac{N_{e,x}}{N_e}, \quad (19)$$

where  $N_{e,x}$  is the number of individuals surviving to age  $x$  out of  $N_e$ . If the hatch rate of eggs laid by females at different ages is the same as the parent cohort, then the hatch rate ( $h_x$ ) and hatchable eggs ( $H_x$ ) at time  $x$  can be calculated as follows:

$$h_x = \frac{H_x}{E_x} = \frac{N_e}{N} \quad (20)$$

$$H_x = E_x \times \frac{N_e}{N}. \quad (21)$$

where  $H_x$  is the number of hatchable eggs among  $E_x$ . In this case, the age-specific fecundity is calculated as follows:

$$m_0 = \frac{0}{N_e}, m_1 = \frac{0}{N_{e,1}}, \dots, m_x = \frac{H_x}{N_{e,x}}. \quad (22)$$

The net reproductive rate based on hatched eggs  $R_{0,e}$  is then:

$$R_{0,e} = \sum_{x=0}^{\infty} l_x m_x = \sum_{x=0}^{\infty} \frac{N_{e,x}}{N_e} \times \frac{H_x}{N_{e,x}} = \sum_{x=0}^{\infty} \frac{N_{e,x}}{N_e} \times \frac{1}{N_{e,x}} \left( E_x \times \frac{N_e}{N} \right) = \sum_{x=0}^{\infty} \frac{E_x}{N}. \quad (23)$$

It is evident that if the hatch rate of eggs laid by females at different ages is the same as that of the parent cohort, the net reproductive rates calculated using all eggs and hatched eggs will be the same; thus,

$$R_{0,e} = \sum_{x=0}^{\infty} \frac{E_x}{N} = R_0. \quad (24)$$

It is easy to prove that the same intrinsic rate of increase and finite rate will be obtained in both cases.

On the other hand, if the hatch rate of eggs laid by females at different ages is not the same as that of the parent cohort, that is:

$$h_x = \frac{H_x}{E_x} \neq \frac{N_e}{N}, \quad (25)$$

then the net reproductive rate based on hatched eggs  $R_{0,e}$  will be as follows:

$$R_{0,e} = \sum_{x=0}^{\infty} l_x m_x = \sum_{x=0}^{\infty} \frac{N_{e,x}}{N_e} \times \frac{H_x}{N_{e,x}} = \sum_{x=0}^{\infty} \frac{H_x}{N_e}. \quad (26)$$

Therefore,

$$R_{0,e} = \sum_{x=0}^{\infty} \frac{H_x}{N_e} \neq \sum_{x=0}^{\infty} \frac{E_x}{N} = R_0. \quad (27)$$

The above discussion proves that if the egg hatch rate varies with maternal age, using the total number of eggs in the calculation of population parameters (i.e.  $r$ ,  $\lambda$ ,  $R_0$  and  $T$ ) will generate inaccurate and overestimated results. Because only the daily hatchable eggs ( $H_x$ ) represent the true fecundity of a female at age  $x$  and  $N_{e,x}/N_e$  represents the true survival rate of hatched eggs at age  $x$ , we recommend

using hatched eggs to calculate the population characteristics.

When all eggs were included in the life table analysis, the adult pre-oviposition period (APOP) and total pre-oviposition period (TPOP) were 8.5 and 28.0 days, respectively (table 2). This APOP is shorter than the results of *H. dimidiata* fed on *Brevicoryne brassicae* (11.04 days, Gillani et al. 2007). Because hatching did not occur in eggs laid during the first 4 days of egg production, coupled with the low hatch rate (<0.1) observed from day 5 to 7 of egg production (fig. 2b), the APOP (16.1 days) and TPOP (35.7 days) of *H. dimidiata* calculated using only hatched eggs were significantly longer and more realistic than those calculated using all eggs. Lewontin (1965) pointed out the importance of the first reproduction age plays to the intrinsic rate. He showed that 1 day saved in development is worth a doubling of fecundity in *Calandra oryzae*. Gabre et al. (2005) showed the mean TPOP for *C. megacephala* was 18.9 days which closed to the age of peak of reproductive value. Huang and Chi (2012) showed also that TPOP can reveal the effect of the first reproductive age on the intrinsic rate and finite rate as well. These findings all support the use of hatched eggs in demographic analysis.

Jha et al. (2012a,b) pointed out that inclusion of unhatched eggs in life table analysis would shift the  $l_x$  curve downward and the  $m_x$  curve upward; in addition, it led to an erroneous relationship between  $R_0$  and  $F$ . They suggested excluding unhatched eggs to obtain realistic demographic characteristics and avoid overestimating population parameters. Our results reinforce their conclusion that when the hatch rate varies with maternal age, the unhatched eggs should be excluded from the life table analysis to remove the influence of the hatch rate of the parent cohort due to sampling.

#### Predation rate of *Harmonia dimidiata*

As noted in other studies (Sahayaraj and Paulraj 2001; Chi and Yang 2003; Mo and Liu 2007; Yu et al. 2013a), significant variations in predation rate due to age and developmental stage were also observed in this study. The problems and subsequent errors attributable to ignoring age and stage structure in predation rate analysis have been previously discussed by Chi and Yang (2003). Moreover, Farhadi et al. (2011) indicated that predation capacity would be overestimated when the female age-specific life table approach is used. All of these studies demonstrate the necessity of including age and stage structure to accommodate these variable predation rates. This can

only be satisfactorily achieved using the age-stage, two-sex life table.

In this study, we supplied *H. dimidiata* with aphids of mixed stages. From the point of view of the predator, the size of the aphids does matter. Due to the limited time and manpower, we provided more aphids than the ladybird beetles could consume. In this way, the survival, development and reproduction of *H. dimidiata* could be sustained. The daily consumption rate  $c_{xj}$ , however, showed higher variation (error bar of SD in fig. 5b), because if one predator consumes 10 fourth instars, another predator may need to consume 40 first instars to obtain an equivalent amount of food. To obtain a precise quantification of predation rate, we suggest supplying *H. dimidiata* with aphids of the same stage. It would then be possible to detect the equivalent coefficient of different stages; that is, a first instar prey may be equivalent to 0.1 of a fourth instar. Using this technique would produce a more precise predation rate.

Dynamics of the predation rate of *H. dimidiata* in our results followed the same pattern as those obtained by Yu et al. (2013a). The net predation rate ( $C_0$ ) in our study was calculated via two approaches, that is by including all eggs and including only hatched eggs. When all 84 eggs were included in the calculation, the  $C_0$  was as low as 9631 prey/predator, due to the inclusion of the unhatched eggs. In contrast, a  $C_0$  as high as 14 804 prey/predator was obtained when only hatched eggs were included. This  $C_0$ , calculated using Eqn 10, exactly equals the mean consumption rate obtained for viable *H. dimidiata* individuals during their lifetime, when only hatched eggs were included (table 2). This realistic consumption value is crucial in order to generate a precise estimation of the number of predators to be released in a biological control procedure.

In this study, the finite predation rate of 125.7 for *H. dimidiata* (table 2) was lower than the 177.0 reported by Yu et al. (2013a). However, Yu et al. (2013a) supplied 800 aphids per plastic cup (5 cm in diameter and 7-cm height), resulting in a density of approximately 40.7 aphids/cm<sup>2</sup>, while in our study, prey density was only 10 aphids/cm<sup>2</sup>. The lower predation rate found in our study may be attributable to lower prey density.

Agarwala et al. (2009) reported that adult female *H. dimidiata* consumed a total of 2244.17 *Cervaphis quercus* aphids at a density of 100 aphids/female/150 cm<sup>2</sup>, which is lower than the predation rate of 13 996.3 *A. gossypii* found in this study. The difference could be a reflection of the lower prey density and also could be due to the different prey species used.

Male *Harmonia axyridis* and *Coccinella septempunctata* consumed an average of 26.2 and 18.8 *Aphis citricola* per day (Lucas et al. 1997), far fewer than the 218.35 melon aphids per day consumed by adult *H. dimidiata* in the present study. The  $Q_p$  values of *Lemnia biplagiata* reared on *A. gossypii* and *P. japonica* on *M. persicae* were 10.4 and 17.7 aphids per predator egg, respectively (Chi and Yang 2003; Yu et al. 2005), which are much less than the number found in our study (219.1 prey/predator offspring). Our results conclusively show that *H. dimidiata* can be an effective biological control agent.

### Computer simulation

Results of our predation study based on life table data showed, as would be expected, there are two non-predatory stages (egg and pupal stages) in *H. dimidiata*'s life history. In our simulation study utilizing 10 eggs (aged 3 days) as the initial population, a non-predatory gap occurred in the predation capacity curve when the beetles entered the pupal stage. This gap, caused by the non-predatory pupal stage, could be compensated for by simulating a timely second release of ladybird beetles. Our data demonstrate the potential value of combining predation studies based on life tables with computer simulation as an invaluable tool for timing the release of predators/parasites in biological control. If we had included all eggs (hatched and unhatched) in our population projection, we would have erroneously overestimated the population size, as well as the predation capacity. These results emphasize the need to exclude unhatched eggs from life table analysis and population projection. Population projection, however, remains a hypothetical numerical simulation without the potential influences of numerous multiple environmental factors. Ideally, supplemental studies of the predator-prey relationship under more realistic field conditions, for example in cages or greenhouses, will be conducted before *H. dimidiata* can be employed to its maximum effectiveness as a biological control agent against *A. gossypii*.

### Conclusion

Our study confirms that the age-stage, two-sex life table can accurately and reliably describe population growth, stage differentiation and predation of a predator population. However, when a species egg hatch rate varies with maternal age, it is essential to exclude the unhatched eggs in the analysis in order to generate accurate demographic parameters. Our results demonstrate that *H. dimidiata* can potentially be

employed as an effective biological control agent of *A. gossypii* aphids, but before doing so, a thorough understanding of the predator–prey relationship obtainable only through life table study and, ideally, supplemented with computer simulation is necessary if any degree of success is to be expected.

### Acknowledgements

We would like to acknowledge the contributions of the reviewers and editor for their valuable comments and suggestions, all of which were instrumental in improving this study. We thank Jih-Zu Yu and his laboratory members for supplying ladybird beetles and for their assistance in plant cultivation and aphid rearing. We thank Prof. Lu-Hung Chen for his help in statistics. This study was partially supported by MOST 102-2313-B-005-028 and MOST 103-2923-B-005-003.

### References

- Agarwala BK, Singh TK, Lokeshwari RK, Sharmila M, 2009. Functional response and reproductive attributes of the aphidophagous ladybird beetle, *Harmonia dimidiata* (Fabricius) in oak trees of sericultural importance. *J Asia-Pac Entomol*, 12, 179–182.
- Birch LC, 1948. The intrinsic rate of natural increase of an insect population. *J Anim Ecol*, 17, 15–26.
- Brandstätter E, 1999. Confidence intervals as an alternative to significance testing. *Math Psychol Res*, 4, 33–46.
- Carey JR, 1993. *Applied demography for biologists with special emphasis on insects*. Oxford University Press, New York.
- Chen SP, Wang CL, Chen CN, 2009. A list of natural enemies of insect pests in Taiwan. Taiwan Agricultural Research Institute, COA., Bureau of Animal and Plant Health Inspection and Quarantine, COA., National Taiwan University, Taiwan.
- Chi H, 1988. Life-table analysis incorporating both sexes and variable development rate among individuals. *Environ Entomol*, 17, 26–34.
- Chi H, 1990. Timing of control based on the stage structure of pest populations: a simulation approach. *J Econ Entomol*, 83, 1143–1150.
- Chi H, 2013a. TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. <http://140.120.197.173/Ecology/>. [accessed on 11 November 2014].
- Chi H, 2013b. CONSUME-MSChart: a computer program for predation rate study based on age-stage, two-sex life table. <http://140.120.197.173/Ecology/>.
- Chi H, 2013c. Timing-MSChart: computer program for population projection based on age-stage, two-sex life table. <http://140.120.197.173/Ecology/>.
- Chi H, Liu H, 1985. Two new methods for the study of insect population ecology. *Bull Inst Zool Acad Sin*, 24, 225–240.
- Chi H, Su HY, 2006. Age-stage, two-sex life tables of *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae) and its host *Myzus persicae* (Sulzer) (Homoptera: Aphididae) with mathematical proof of the relationship between female fecundity and the net reproductive rate. *Environ Entomol*, 35, 10–21.
- Chi H, Yang TC, 2003. Two-sex life table and predation rate of *Propylaea japonica* Thunberg (Coleoptera: Coccinellidae) fed on *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Environ Entomol*, 32, 327–333.
- Chi H, Mou DF, Allahyari H, Yu JZ, Huang YB, Yang TC, Farhadi R, Gholizadeh M, 2011. Finite predation rate: a novel parameter for the quantitative measurement of predation potential of predator at population level. In: *Nature precedings*. <http://hdl.handle.net/10101/npre.2011.6651.1>.
- Davidson J, 1944. On the relationship between temperature and rate of development of insects at constant temperatures. *J Anim Ecol*, 13, 26–38.
- Efron B, Tibshirani RJ, 1993. *An introduction to the bootstrap*. Chapman & Hall, New York.
- Farhadi R, Allahyari H, Chi H, 2011. Life table and predation capacity of *Hippodamia variegata* (Coleoptera: Coccinellidae) feeding on *Aphis fabae* (Hemiptera: Aphididae). *Biol Control*, 59, 83–89.
- Fox CW, 1993. The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Oecologia*, 96, 139–146.
- Gabre RA, Adham FK, Chi H, 2005. Life table of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). *Acta Oecol*, 27, 179–183.
- Gillani WA, Matin MA, Rafi MA, 2007. Bionomics of tropical coccinellid beetle *Harmonia (Leis) dimidiata* (F) (Coleoptera: Coccinellidae) under laboratory conditions. *Pakistan J Agric Res*, 20, 79–83.
- Goodman D, 1982. Optimal life histories, optimal notation, and the value of reproductive value. *Am Nat*, 119, 803–823.
- Huang YB, Chi H, 2012. Age-stage, two-sex life table of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) with a discussion on the problem of applying females age-specific life table to insect populations. *Insect Sci*, 19, 263–273.
- Jha RK, Chi H, Tang LC, 2012a. A comparison of artificial diet and hybrid sweet corn for the rearing of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) based on life table characteristics. *Environ Entomol*, 41, 30–39.
- Jha RK, Chi H, Tang LC, 2012b. Effects of survival rate and fecundity on the intrinsic rate of increase of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Formosan Entomol*, 32, 223–235.

- Leslie PH, 1945. On the use of matrices in certain population mathematics. *Biometrika*, 33, 183–212.
- Leslie PH, 1948. Some further notes on the use of matrices in population mathematics. *Biometrika*, 35, 213–245.
- Lewis EG, 1942. On the generation and growth of a population. *Sankhya*, 6, 93–96.
- Lewontin RC, 1965. Selection for colonizing ability. In: *The genetic of colonizing species*. Ed. by Baker HG, Stebbins GL, Academic Press, San Diego, CA, 77–94.
- Livdahl TP, Edgerly JS, 1987. Egg hatching inhibition: field evidence for population regulation in a treehole mosquito. *Ecol Entomol*, 12, 395–399.
- Lotka AJ, 1907. Studies on the mode of growth of material aggregates. *Am J Sci*, 24, 199–216.
- Lucas É, Coderre D, Vincent C, 1997. Voracity and feeding preferences of two aphidophagous coccinellids on *Aphis citricola* and *Tetranychus urticae*. *Entomol Exp Appl*, 85, 151–159.
- Messenger PS, 1964. Use of life tables in a bioclimatic study of an experimental aphid-braconid wasp host-parasite system. *Ecology*, 45, 119–131.
- Mo TL, Liu TX, 2007. Predation and life table of *Feltiella acarisuga* (Diptera: Cecidomyiidae) preying on eggs of *Tetranychus urticae* (Acari: Tetranychidae). *Environ Entomol*, 36, 369–375.
- Perry JC, Roitberg BD, 2005. Ladybird mothers mitigate offspring starvation risk by laying trophic eggs. *Behav Ecol Sociobiol*, 58, 578–586.
- Sahayaraj K, Paulraj MG, 2001. Rearing and life table of reduviid predator *Rhynocoris marginatus* Fab. (Het., Reduviidae) on *Spodoptera litura* Fab. (Lep., Noctuidae) larvae. *J Appl Entomol*, 125, 321–325.
- Tao CC, 1990. *Aphis gossypii* Glover. In: *Aphis-fauna of Taiwan province, China*. Ed. by Tao CC, Taiwan Museum, Taipei, Taiwan, 176–177.
- Tuan SJ, Lee CC, Chi H, 2013. Population and damage projection of *Spodoptera litura* (F.) on peanuts (*Arachis hypogaea* L.) under different conditions using the age-stage, two-sex life table. *Pest Manag Sci*, 70, 805–813.
- Yang TC, 1994. Demographic analysis and computer simulation of the predator-prey relationship between *Propylaea japonica* Thunberg and *Myzus persicae* (Sulzer). Masters Thesis, Department of Entomology, National Chung Hsing University, Taichung, Taiwan.
- Yu JZ, Chi H, Chen BH, 2005. Life table and predation of *Lemnia bipagiata* (Coleoptera: Coccinellidae) fed on *Aphis gossypii* (Homoptera: Aphididae) with a proof on relationship among gross reproduction rate, net reproduction rate, and preadult survivorship. *Ann Entomol Soc Am*, 98, 475–482.
- Yu JZ, Chi H, Chen BH, 2013a. Comparison of the life tables and predation rates of *Harmonia dimidiata* (F.) (Coleoptera: Coccinellidae) fed on *Aphis gossypii* Glover (Homoptera: Aphididae) at different temperatures. *Biol Control*, 64, 1–9.
- Yu LY, Chen ZZ, Zheng FQ, Shi AJ, Guo TT, Yeh BH, Chi H, Xu YY, 2013b. Demographic analysis, a comparison of the jackknife and bootstrap methods, and predation projection: a case study of *Chrysopa pallens* (Neuroptera: Chrysopidae). *Entomol Soc Am*, 106, 1–9.