

# Life-history traits of the predator *Rhyzobius lophanthae* reared on the scale *Chrysomphalus dictyospermi*

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## Abstract

*Rhyzobius lophanthae* Blaisdell (Coleoptera Coccinellidae) is a coccidophagous predator known as a natural enemy of Diaspididae scales. This study aimed to determine the suitability of *Chrysomphalus dictyospermi* Morgan (Hemiptera Diaspididae) as prey for this predator. Several traits such as larval development and survival, reproductive performance and reproductive investment were assessed. Pre-imaginal development time was short whereas immature age-specific survival rate was high. Fecundity, expressed in terms of lifetime fecundity and oviposition rate, was high. The soma and gonads fat contents of females were estimated to be  $86.67 \pm 1.52$  and  $13.33 \pm 1.52\%$ , respectively. Reproductive effort, the total egg mass expressed as a proportion of the female initial fresh mass, were almost 9-fold of their mass in eggs. Overall, our results show that *C. dictyospermi* is an essential and highly suitable prey for *R. lophanthae* and allows a better performance than other prey species used in previous studies.

**Key words:** coccidophagous ladybird, armored scale, life-history traits, reproductive investment, prey suitability.

## Introduction

The study of the life-history traits of the organisms is of paramount importance for applied and fundamental ecology. The traits not only characterize the biology and ecology of the organisms but also they are components of the fitness and its variability mediate natural selection (Partridge and Harvey, 1988; Partridge and Sibly, 1991; Roff, 2002; Stearns, 2004).

*Rhyzobius lophanthae* Blaisdell (Coleoptera Coccinellidae) is a coccidophagous ladybird predator of the subfamily Coccidulinae and tribe Coccidulini (Raimundo and Alves, 1986), with a worldwide distribution (Kovář, 2007) and it is known as a natural enemy of armored scale species (Hemiptera Diaspididae) (Stathas, 2000; 2001; Stathas *et al.*, 2002). This species is native to Australia but it was deliberately introduced in several regions of the globe as biological control agent (Yus, 1973; Raimundo and Alves, 1986). It has been reported as an effective biocontrol agent in California, Italy (Honda and Luck, 1995; Stathas, 2000), Morocco (Smirnov, 1950; Honda and Luck, 1995), Israel, East Africa and Hawaii (Honda and Luck, 1995). The success of this predator is attributed to its biological characteristics; specificity for Diaspididae, fecundity, and adult longevity as well its ecological characteristics; lack of diapause, high mobility, rapid population growth, high number of generations per year and lack of parasitism (Stathas, 2000; Vives, 2002). The biology and ecology of *R. lophanthae* are poorly known. Smirnov (1950), observed that *Chrysomphalus dictyospermi* Morgan (Hemiptera Diaspididae) was a suitable prey for the reproduction of the predator but did not tested prey suitability for any other life-history traits. Prey suitability strongly differs, affecting differently several life-history traits (Hodek and Evans, 2012). Types of food are (i) essential, when ensure the completion of life cy-

cle and oviposition or (ii) alternative, when serve only as a source of energy and thus prolong survival in comparison with starvation. There are transitions between those groups, and essential food show as well varying degrees of favourability, enabling different developmental rates, fecundity and survival (Rana *et al.*, 2002; Soares *et al.*, 2004; Evans, 2005; Evans and Gunther, 2005; Michaud, 2005; Sebastião *et al.*, 2015).

*R. lophanthae* is present in the Azores (Portugal) (Soares *et al.*, 2003; 2006). This species, as most ladybird species of the Azorean fauna, is small sized (Soares *et al.*, 2017; Honěk *et al.*, 2017) and thus it is expected to have a smaller voracity. Larger ladybirds, such as *Harmonia axyridis* Pallas (Coleoptera Coccinellidae) (Soares *et al.*, 2004), are more voracious than medium size species, e.g. *Coccinella undecimpunctata* L. (Coleoptera Coccinellidae) (Cabral *et al.*, 2006; 2009) and even more than the smaller *Scymnus* spp. (Borges *et al.*, 2011; 2013; Sebastião *et al.*, 2015). The role of smaller ladybirds in biological control, however, should not be underestimated once they can be efficient (i) at earlier and later developmental stages of prey colonies when prey abundance is low and (ii) because they do not consume the totality of the prey and thus the killing potential of the predator is enhanced (Sebastião *et al.*, 2015). The present study aimed to determine life-history traits of the immature and adult stages, as well as the reproductive investment, of an Azorean population of *R. lophanthae* fed on *C. dictyospermi*.

## Materials and methods

### Biological material

*R. lophanthae* adults were collected in Fenais da Luz, on São Miguel Island (Azores, Portugal) from *Acacia melanoxylon* R.Br. (Fabales Fabaceae) infested with the

black scale *Lindingaspis rossi* Maskell (Hemiptera Diaspididae). The predator was maintained at  $25 \pm 1$  °C,  $75 \pm 5\%$  RH and under a fluorescent (Philips ref.: TDL 23W/54 and TDL 18W/54) light regime of 16L: 8D (Smirnoff, 1950; Cividanes and Gutierrez, 1996; Stathas, 2000; 2001; Stathas *et al.*, 2002). Preliminary tests revealed that *L. rossi* was a poor quality prey and for this reason, *C. dictyospermi* on *Strelitzia reginae* Banks (Zingiberales Strelitziaceae) was provided as food resource.

#### Predator life-history traits

Field collected adults of *R. lophanthae* were coupled and isolated in 5 cm diameter  $\times$  2 cm height plastic boxes and fed *ad libitum* with *C. dictyospermi*. Couples were observed every day at 11 AM. The host plant leaves were carefully checked for the presence of eggs that are generally laid underneath the scale shield. The eggs were removed and isolated individually in similar boxes to determine the incubation period. The newly hatched larvae were fed *ad libitum* with *C. dictyospermi* and daily observations were carried out in order to determine larval developmental traits. Each larva was observed daily in order to determine the duration of larval stages and the larval survival. The newly emerged adults were weighed and the sex-ratio determined. Sexual determination is an easy process once males and females differ in the configuration of last sternite: arched in females and with a recess like open wide U in males (Stathas *et al.*, 2002).

Reproductive performance of *R. lophanthae* was assessed through pre-oviposition period, reproductive life-span, life-time fecundity and daily oviposition rate of females. Total life-span of females was also estimated. One couple per plastic box fed *ad libitum* with *C. dictyospermi*, was kept till the death of the individuals. To determine the fertility, a test was done for 5 days with 10 females around the 30<sup>th</sup> day of oviposition. The number of hatched larvae, embryonated and infertile eggs were recorded in order to calculate a fertility rate ( $N = 943$ ).

To determine the larval survival under starvation, 14 larvae were isolated individually in plastic boxes and fed *ad libitum* until the 4<sup>th</sup> larval stage. When larvae moulted into the 4<sup>th</sup> instar, larvae were deprived of food. The larvae were observed twice a day to determine the survival time. A similar experiment was performed with 14 days old females.

*C. dictyospermi* was provided every day on a piece of heavily infested *S. reginae* leaf (roughly with the size of the plastic boxes). Because fresh leaf pieces were provided every day, the host plant kept turgid and prey alive. All these experiments were performed at  $25 \pm 1$  °C,  $75 \pm 5\%$  RH and under a fluorescent (Philips ref.: TDL 23W/54 and TDL 18W/54) light regime of 16L: 8D.

The methodology to determine fat content in soma and gonads was based in Magro *et al.* (2003). Pieces of aluminum foils with approximately  $1 \times 1$  cm were washed in cyclohexane and dried in the oven at 35 °C. Females were dissected upon a piece of aluminum foil in a drop

of distilled water. Gonads were separated from the soma, cut off and placed in another aluminum foil containing a drop of distilled water. After dissection, the soma and gonads were dried in oven at 35 °C overnight and weighed twice a day on a Sartorius ultra-microbalance SE 2 until mass had stabilized (mass variation  $< 0.001$  mg). After weight stabilization, the soma and gonads were submerged in cyclohexane during 9 h to extract fat. After this first fat extraction cycle, the solvent contained in the soma and gonads was allowed to evaporate off overnight. A second fat extraction was performed in the next day and the mass of each one was recorded. The proportions of fat in the soma and the gonads were calculated as follows:

Soma fat proportion =  $(S_{wi} - S_{wf}) / (S_{wi} - S_{wf}) + (G_{wi} - G_{wf})$ ;  
Gonad fat proportion =  $(G_{wi} - G_{wf}) / (S_{wi} - S_{wf}) + (G_{wi} - G_{wf})$ ;

where  $S_{wi}$  and  $S_{wf}$  are respectively the mass of the soma before and after fat extraction,  $G_{wi}$  and  $G_{wf}$  are respectively the mass of the gonads before and after fat extraction.

#### Reproductive investment

To determine the reproductive investment, in terms of egg mass production, an experiment was carried out in which 14 ovipositing females aged from 15 to 30 days old. The couples were isolated in 5 cm diameter  $\times$  2 cm height plastic boxes and maintained at  $25 \pm 1$  °C,  $75 \pm 5\%$  RH and under a light regime of 16L: 8D and fed *ad libitum* with *C. dictyospermi*. Every day, 4 eggs per female were randomly collected ( $N = 280$ ). Each egg was weighted on a Sartorius ultra-microbalance SE 2 and photographed to determine the length (L) and the width (W) using the software ImageJ 1.47t (Rasband, 2013). The volume of each egg was calculated as follows (Vargas *et al.*, 2012):

$$\text{Egg volume} = LW^2\pi/6$$

Reproductive effort was calculated as the total egg mass expressed as a proportion of the female initial fresh mass (Vargas *et al.*, 2012):

Reproductive effort =  $(\text{fecundity} \times \text{egg mass}) / \text{adult mass}$ ;  
where the fecundity corresponds to lifetime fecundity (number of eggs), egg mass is expressed in mg and adult mass corresponds to initial female mass (mg).

#### Statistical analysis

Data normality of the development time of immature stages was assessed with the Kolmogorov–Smirnov test. Because data were not normal, a non-parametric test was adopted. To compare the developmental time of immature stages, the Kruskal–Wallis test was performed. Post-hoc pairwise comparisons were made using the Mann–Whitney with the Bonferroni correction. A *t*-test was used to compare the weight of males and females, after confirming normality of data with the Kolmogorov–Smirnov test. To compare longevity of larvae and females under starvation, data normality and variance homogeneity were assessed by the Kolmogorov–Smirnov and Levene’s tests, respectively. Due to the non-homogeneity of variances, the Mann–Whitney test was performed. SPSS v. 15.0 was used to perform statistical analysis (SPSS, 2006).

**Table 1.** Duration of embryonic development and immature stages (mean  $\pm$  SE) and survival (%) of *R. lophanthae* reared on *C. dictyospermi* ( $25 \pm 1$  °C,  $75 \pm 5\%$  RH and a light regime of 16L: 8D).

Pre-imaginal stages	Days (mean $\pm$ SE)	Age-specific survival rate (%)	Accumulated age specificity survival rate (%)
Egg	5.55 $\pm$ 0.11 a	70.52 $\pm$ 0.01	100
1 <sup>st</sup> instar	3.73 $\pm$ 0.22 b	88.47 $\pm$ 0.06	70.52 $\pm$ 0.09
2 <sup>nd</sup> instar	2.64 $\pm$ 0.12 c	100	59.01 $\pm$ 0.10
3 <sup>rd</sup> instar	2.55 $\pm$ 0.14 c	96.15 $\pm$ 0.04	59.01 $\pm$ 0.10
4 <sup>th</sup> instar	5.68 $\pm$ 0.29 a	100	55.16 $\pm$ 0.10
Pupa	4.64 $\pm$ 0.15 d	100	55.16 $\pm$ 0.10

Within a column means followed by different letters are significantly different at  $p < 0.05$  (Kruskal-Wallis test).

**Table 2.** Life-history traits (mean  $\pm$  SE) of *R. lophanthae* fed on *C. dictyospermi* under constant abiotic conditions:  $25 \pm 1$  °C,  $75 \pm 5\%$  RH and a light regime of 16L: 8D. N- Number of replicates.

Life-history traits	N	<i>R. lophanthae</i> (mean $\pm$ SE)
Sex ratio (% of females)	22	45.45 $\pm$ 0.11
Adult body weight (mg)	12	2.00 $\pm$ 0.11 a
	10	2.45 $\pm$ 0.19 b
Pre-oviposition period (days)	9	4.22 $\pm$ 0.32
Total adult life-span(days)	9	66.00 $\pm$ 3.70
Reproductive life-span (days)	9	58.30 $\pm$ 4.32
Lifetime fecundity (eggs)	9	891.00 $\pm$ 116.61
Oviposition rate (eggs/day)	9	15.05 $\pm$ 1.34
Soma fat content	14	86.67 $\pm$ 1.52
Gonads fat content (%)	14	13.33 $\pm$ 1.52
Longevity under starvation (days)	14	10.07 $\pm$ 0.67 a
	14	3.43 $\pm$ 0.41 b

For longevity under starvation and adult body weight, means followed by different letters are significantly different at  $p < 0.05$  (Mann-Whitney and t-test, respectively).

**Table 3.** Reproductive investment expressed in terms of egg mass production and reproductive effort (mean  $\pm$  SE) of *R. lophanthae* fed on *C. dictyospermi* under constant abiotic conditions:  $25 \pm 1$  °C,  $75 \pm 5\%$  RH and a light regime of 16L: 8D.

Reproductive investment	Mean $\pm$ SE
Egg mass (mg)	0.024 $\pm$ 0.001
Egg length (mm)	0.514 $\pm$ 0.005
Egg width (mm)	0.254 $\pm$ 0.002
Egg volume (mm <sup>3</sup> )	0.018 $\pm$ 0.001
Reproductive effort	8.728 $\pm$ 0.614

Reproductive effort = (fecundity  $\times$  egg mass)/adult mass.

## Results

### Predator life-history traits

There were significant differences in the developmental time of larval stages of *R. lophanthae* (Kruskal-Wallis test:  $\chi^2 = 94.17$ ,  $df = 5$ ,  $p < 0.001$ ) (table 1). The total pre-imaginal (from egg to adult) development time was  $24.82 \pm 0.36$  days. The developmental time of the 4<sup>th</sup> instar was significantly longer than the other larval instars. The 2<sup>nd</sup> instar and the 3<sup>rd</sup> instar larvae also took the same time to develop and are the fastest stages (table 1). Average larval survival was high,  $84.60 \pm 0.07\%$  (table 1).

Concerning the sex-ratio, the proportion of females was  $45.45 \pm 0.11\%$ . Females are heavier than males ( $t$  test:  $t = 2.130$ ,  $df = 20$ ,  $p = 0.046$ ). The pre-oviposition period is roughly  $4.22 \pm 0.32$  days. Females lived approximately  $66.00 \pm 3.70$  days and laid eggs for  $58.30 \pm 4.32$  days producing an average of  $891.00 \pm 116.61$  eggs at a rate of  $15.05 \pm 1.34$  eggs/day. The soma and gonads fat contents of females were estimated to be  $86.67 \pm 1.52$  and  $13.33 \pm 1.52\%$ , respectively. When individuals were submitted to starvation, larvae survived longer than adult females ( $U = 1.500$ ,  $df = 26$ ,  $p \leq 0.0001$ ) (table 2).

### Reproductive investment

The eggs weighed  $0.024 \pm 0.001$  mg with the dimensions  $0.514 \pm 0.005$  mm  $\times$   $0.254 \pm 0.002$  mm. The egg volume was about  $0.018 \pm 0.001$  mm<sup>3</sup>. Proportionally to their body weight, females invest almost 9-fold of their mass in eggs (table 3).

## Discussion

Our preliminary tests revealed that *L. rossi* was less suitable than *C. dictyospermi*; survival of larvae was less than 20% and females laid only one or two eggs every day. Comparing with previous studies under similar laboratory conditions (Cividanes and Gutierrez, 1996;

Stathas, 2000; 2001; Stathas *et al.*, 2002), *C. dictyospermi* seems to be more suitable for *R. lophanthae*. The duration of pre-imaginal development (from egg to adult) is lower (24.82 days) than when fed on other species of scales, for example: 28.30 days on *Chrysomphalus aonidium* L. (Hemiptera Diaspididae) (Stathas *et al.*, 2002); 36 days on *Aspidiotus nerii* Bouche (Hemiptera Diaspididae) (Stathas, 2000); 27.10 on *A. neri* and 48.80 days on *Aonidiella aurantii* Maskell (Hemiptera Diaspididae) (Stathas, 2001). However, mortality of larval stages is higher on *C. dictyospermi* (15.38%) than on *C. aonidium* (9%) (Stathas *et al.*, 2002) and on *A. nerii* (0%) (Stathas, 2001). The daily oviposition rate on *A. nerii* was higher than with *C. dictyospermi* (18-20 eggs/day vs 15.05 eggs/day) (Cividanes and Gutierrez, 1996; Stathas, 2000), but the total fecundity was higher on the latter (633.7 eggs vs 891.0 eggs) (Stathas, 2000). On the other hand, longevity is similar (63.4 days vs 66.0 days, respectively) (Stathas, 2000).

Lifetime fecundity of *R. lophanthae* females was high. According to Rosen (1990), lifetime fecundity of more than 600 eggs per female is high for coccidophagous predators. Females present a rate of fertility of  $70.52 \pm 0.09\%$ , during the test for 5 days. According to Michaud (2005), measurements of the fecundity and fertility produced by females over a fixed period in early adult life is likely sufficient to assess relative prey suitability. Aphidophagous female ladybirds are known to lay trophic eggs, that is, infertile eggs that become the first meal of newly hatched larvae. This behaviour is considered adaptative because it enhances the survival chances of newborn larvae. We believe that the rate of  $29.48 \pm 0.09\%$  of infertile eggs represents a considerable resource investment in reproduction to be wasted with no return in terms of fitness. Thus this result leads us to question if coccidophagous ladybird could have evolved a similar behaviour as the aphidophagous species. Further studies are required to test this hypothesis because for other coccidophagous species such as *Nephus reunioni* Fursch (Coleoptera Coccinellidae) only 7.65% of the eggs are infertile (Borges *et al.*, 2013).

How organisms allocate fat to gonads and soma is commonly used to determine reproductive investment in insects (Herz and Heitland, 2002; Magro *et al.*, 2003; Borges *et al.*, 2011). Reproductive effort, which is positively correlated with fecundity, is expected to be lower in coccidophagous than aphidophagous. For instance, reproductive effort of *R. lophanthae* ( $8.7 \pm 0.6$ ) is lower than the aphidophagous *Coleomegilla maculata* De Geer ( $14.3 \pm 1.5$ ) (Vargas *et al.*, 2012). This fact seems to be the result of natural selection driven by the availability of food resources in space and time. Field data reveal that, contrarily to aphids, coccids form smaller colonies, more aggregated in space but available for longer periods of time (Borges *et al.*, 2006; 2011). This factor forces coccidophagous females to move more often between prey colonies and their larvae to adapt a very sparse food resource. We found that longevity of larvae under starvation was higher for 4<sup>th</sup> instar larvae than for females (10.0 vs. 3.9 days). The result obtained for females in our study is similar to the one obtained by Rosmarina (1983) for *R. lophanthae* females (3.5 days).

Different factors may account for the survival of the young larvae, including the selection of the most suitable oviposition sites, which are under maternal control, and the size of the newly-hatched larvae, which in turn is positively related to egg size (Nedvěď and Honěk, 2012). Coccinellid egg size is arguably held close to a minimum determined by the ability of newly-hatched larvae to capture their first prey item (Vargas *et al.*, 2012). *R. lophanthae* egg dimensions are similar to ones determined by Stathas (2001) (length:  $0.51 \pm 0.01$  vs.  $0.50 \pm 0.02$  mm and width:  $0.25 \pm 0.002$  vs.  $0.25 \pm 0.01$  mm). Unlike other traits of *R. lophanthae*, in which some differences to the Azorean populations are notorious, the size of the eggs seems to be similar. In fact, egg size is genetically determined and maintained even under limited food supply conditions (Stewart *et al.*, 1991a; 1991b; Dixon and Guo, 1993).

## Conclusions

In this study the biology and ecology of *R. lophanthae* under a single diet of *C. dictyospermi* was studied, determining life-history traits directly associated with reproduction and survival, which are important components of fitness for applied and fundamental ecology. The results intended to contribute to the evaluation of the performance of *R. lophanthae* on the scale *C. dictyospermi*, a prey that was never tested with this predator. In this way, we found that *R. lophanthae* could be a very good candidate as biocontrol agent against *C. dictyospermi*.

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