

The life table of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) after different storage periods

Mehmet Salih Özgökçe^{1*}, Remzi Atlihan¹ and İsmail Karaca²

¹Yuzuncu Yil University, Agriculture Faculty, Plant Protection Department, Van, Turkey. ²Süleyman Demirel University, Agriculture Faculty, Plant Protection Department, Isparta, Turkey. *e-mail: msozgekce@yyu.edu.tr

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Abstract

The life table of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) was calculated on *Planococcus citri* (Risso) (Homoptera: Coccidae) under laboratory conditions at 25±1°C and 45±5% relative humidity after different storage periods at 15°C of cooling. Experiments consisted of 5 groups including a control and design experiments that were run for 5, 10, 15 and 20 days, respectively. The following predictive parameters were obtained for the control and design experiments: intrinsic rate of increase $r_m = 0.098, 0.092, 0.074, 0.058$ and 0.045 ; net reproductive rate $R_0 = 340.703, 276.629, 149.930, 119.321$ and 65.928 ; mean generation time $T_0 = 59.350, 60.851, 67.726, 83.189$ and 94.173 , respectively. Doubling time $DT = 7.2, 7.9, 9.3, 12.1$ and 15.3 , and finite rate of increment $\lambda = 1.101, 1.092, 1.074, 1.059$ and 1.046 , were calculated as well. The reproductive value (V_x) for each group was plotted.

Key words: *Cryptolaemus montrouzieri*, *Planococcus citri*, life table, fecundity, mortality, storage.

Introduction

Predaceous coccinellids are important biological control agents of pest species, including aphids and adelgids¹⁻³, mealybugs⁴, scales⁵⁻⁷, whiteflies^{8,9}, psyllids⁹ and mites^{4,11,12}. They were widely cited as important biological control programs and may be used as potential biocontrol agents for management of the pest populations¹³.

Cryptolaemus montrouzieri, which is a species of ladybird beetle native to Australia known as the mealybug destroyer, is widely used to control *Planococcus citri* (Risso) (Homoptera: Coccidae) in citrus production areas. They are mass reared in insectariums and transferred to these areas for controlling mealybug at critical periods. This predator is a very efficient natural enemy of mealybugs, with both larvae and adults preying on these pests. They are commonly used in citrus, grapes and greenhouses where mealybugs are major problems. Typical release rates for orchards are 5-10 adults per tree and they are released one or two times per year depending on pest populations. They are considerably effective when mealybug populations are high, and repeated releases may be necessary. Large scale production may be needed to obtain enough predators for the grower to suppress mealybug populations during the critical period. For this reason, storage of predator may be needed, and storage conditions must not damage their performance characteristics such as development, survival and fecundity. A former study indicated that the most suitable temperature for storage of *C. montrouzieri* adults was 15°C, with high survival rate at that temperature¹⁴. This tropical species is inactive at 15-20°C and a minimum temperature of 21°C is needed to feed and lay eggs¹⁵. The reproduction rate is a crucial factor in the population growth. Wittmeyer and Coudron¹⁶ pointed out that life tables and fertility tables were powerful tools for analyzing and understanding the effect of an external impact on the growth, survival, reproduction and rate of increase of an insect population.

In this study, our aim was to determine the best storage time for *C. montrouzieri* adults by studying survival, reproduction and life table parameters.

Materials and Methods

Rearing of prey and predator: Potato tubers, cooled at 7°C for a few days, were placed into environmental chambers and temperature was increased gradually to obtain shoots. Eggs, crawlers and adults of mealybugs were transferred to the tubers with shoots. During this period, laboratory conditions were maintained at a relative humidity of 45±5%, a temperature of 25±1°C, and a 16:8 day time:night time ratio. After the mealybug population had increased on potato shoots adequately, *Cryptolaemus* adults were released on the mealybugs. Adults and eggs of the predator that were used for the experiments were obtained after rearing one generation under those laboratory conditions on all stages of mealybug.

Experiments: Experiments were based on 5 cohorts: the control and design experiments that had been kept at the 15°C during 5, 10, 15, 20 days, respectively. On every cohort, 11-12 pairs newly laid eggs were kept in medium-sized Petri dishes (9 cm diameter x 2 cm height) with relative humidity 45±5%, temperature 25±1°C, and a 16:8 day time:night time ratio conditions. These eggs were not handled directly; they were transferred with a soft brush. After hatching, the larvae were fed with mealybugs to obtain adults. All individual adults of the design experiment cohorts were kept together in commercial cells without food during cooling periods of 15°C. After cooling periods, every cohort was transferred to the 25°C environmental chambers again, and their pairs were released in Petri dishes and fed with mealybugs during the rest of their lives. Mealybugs were replaced daily and eggs laid by females

were counted and recorded daily till all adults died. During the experiments, fecundity and longevity of the adults were also determined daily.

Because *C. montrouzieri* feeds on both the mealybugs and their honeydew, we placed a filter paper saturated by honey with all stages of mealybugs into the Petri dishes. Five cohorts, each consisting of 11-12 pairs, were set up in commercial cells. *C. montrouzieri* adults were visually sexed by examination of the first pairs of legs; they are reddish brown to tallow-colored for males, and they range from grey to black for females¹⁷.

Data analyses and statistics: Effect of different storage periods on the growth of populations of *C. montrouzieri* were assessed by constructing a life table, using age-specific survival rates (l_x) and fecundity (m_x) for each age interval (x) per day¹⁸⁻²⁰, from hatching to death. From the data, the following population parameters were calculated: the mean total number of eggs produced by a predator female, measured in females/female/generation; net reproductive rate ($R_0 = \sum l_x m_x$), number of female daughters produced per female, measured in females/female/generation; intrinsic rate of increase (r_m), which describes the growth potential of a population under a given set of environmental conditions and it is calculated by solving the equation $\sum l_x m_x e^{(-r_m x)} = 1$; finite capacity for increase ($\lambda = e^{r_m}$), the number of times the population will multiply itself per unit of time, measured in females/female/day; mean generation time ($T_0 = \ln R_0 / r_m$), the mean time required for a given population to finish 1 generation, measured in days; doubling time ($DT = \ln 2 / r_m$), and the time required for a given population to double its numbers, measured in days. The reproductive values (V_x) per age class of the females in each group were plotted as fertility (m_x) and survival (l_x) versus age (x).

The differences in intrinsic rate of increase (r_m), were tested for significance by estimating the variance using the jack-knife method which facilitated calculation of the standard errors of r_m estimates²¹.²² The jack-knife pseudo-value r_j was calculated for the n samples using the equation $r_j = n \times r_{all} - (n-1) \times r_i$. The mean values of $(n-1)$ jack-knife pseudo-values for mean growth rate for each treatment were subjected to analysis of variance followed by Tukey-Kramer Multiple Comparison Test ($P < 0.05$). The analyses were conducted by using SAS Institute statistical software²³.

Data were analyzed by using PROC GENMOD in SAS/STAT²³. Because the standard linear model assumptions did not match in our data set, generalized linear models (GLM) were used for data analyses and parameter estimations. The class of generalized linear models is an extension of traditional linear models that allows the mean of a population to depend on a *linear predictor* through a nonlinear *link function* and allows the response probability distribution to be any member of an exponential family of distributions. Gamma, Poisson and Negative Binomial distributions of pre-oviposition, oviposition, post-oviposition periods, longevity, total fecundity, mortality of pairs were tested by using the log link function for the lifetime and the data²⁴. The scaled deviance statistic was used in assessing the goodness of fit for specific models. The $(1-\alpha)100\%$ Wald confidence interval was used for parameters. Least-square means corresponding to the group effects were calculated by the LSMEANS statement.

Results

Mortality rates during storage periods: Mortality rate increased with extended storage period. During the cooling periods survival rates of *C. montrouzieri* adults were measured as 86, 64, 67 and 36% for individuals kept at the storage periods of 5, 10, 15 and 20 days, respectively. Survival rates of individuals stored at 5 days declined gradually with age, while survival rates of individuals stored longer than 5 days declined sharply.

Longevity and fecundity parameters: Data of pre-oviposition, oviposition and post-oviposition periods, female longevity and fecundity are presented in Table 1. They were tested according to Gamma, Negative Binomial and Poisson distribution, and means were compared. After storage period, individuals of all cohorts did not lay egg at the same day when they were transferred to 25°C. The pre-oviposition period increased with increasing storage period and values obtained in 15 and 20 days storage periods were significantly longer than those of other storage periods tested and the control ($P < 0.05$). Keeping in different storage periods did not change the oviposition period of the predator, and no significant differences were obtained among the different cohorts ($P < 0.05$). The post-oviposition periods decreased as storage periods increased and values obtained for control individuals and individuals exposed to a 5 day storage period were significantly higher than those of individuals exposed to 10, 15 and 20 days storage periods ($P < 0.05$). Storage period affected longevity of the predator, and longevity of the control cohorts were significantly greater than those of cohorts kept in different storage periods. However, there were no significant differences among cohorts kept in different storage periods ($P < 0.05$). Highest total fecundity was observed for the control cohort (805.0 eggs) followed by the 5 day cohort (686.7 eggs) and the 10 day cohort (478.5 eggs); they were also significantly different in the 15 day and 20 day cohorts according to the Poisson test ($P < 0.05$). Daily reproduction values obtained for the control (7.0 eggs) and the 5 days cohort (9.8 eggs) were significantly higher than those of 15 day (3.0 eggs) and 20 day (3.1 eggs) cohorts $F = 5.08$; $df = 4, 25$; $P = 4.10^{-2}$). Daily reproduction rates for all cohorts were compared according to the Tukey-Kramer Multiple Comparison Test ($P < 0.05$).

Life table parameters: A horizontal life table was constructed and the predictive population parameters estimated in absence of mortality according to Southwood²⁰. From the horizontal life table, the following data were obtained for each group. Net reproductive rates (R_0) were found as 340.703, 276.629, 149.930, 119.321, 65.928, female per cohort female in a generation for the control, 5, 10, 15, and 20 day storage groups, respectively. In the same order, mean generation times (T_0) were calculated as 59.350, 60.851, 67.357, 83.189, 94.173 days and the intrinsic rates of increase (r_m) obtained were 0.098, 0.092, 0.074, 0.058, 0.045, respectively, (Table 2).

The doubling time and finite rate of increase for each cohort were observed to decrease from the control to the 20 day cohort. According to these results, doubling times (DT) were calculated as 7.2, 7.9, 9.3, 12.1 and 15.3, respectively, and the finite rate of increases (λ) obtained were 1.101, 1.092, 1.077, 1.059 and 1.046 individuals per female per day. Persad and Khan²⁵ determined the following life table parameters for *C. montrouzieri* on *Maconellicoccus hirsutus* Green (Hom.: Pseudococcidae): Innate capacity of increase $r_m = 0.135$, net reproductive rate $R_0 = 227.18$,

Table 1. Oviposition, longevity and fecundity parameters of *Cryptolaemus montrouzieri* after different storage periods at the 15°C degree.

Cohort	n	Pre-oviposition	Oviposition	Post-oviposition	n	Longevity	n	Total fecundity	Daily reproduction
Control	9	5.1±0.64 b ^y (2-8)	109.3±14.61 a (27-150)	5.4±0.73 a (1-11)	9	120.8±17.40 a (39-159)	9	805.3±92.07 a (376-1321)	7.0±0.58 a (4.33-9.64)
5 days	6	4.0±0.52 b (2-5)	56.3±19.51 a (18-109)	5.2±0.90 a (3-8)	6	69.7±21.32 b (19-126)	6	686.7±112.76 a (93-1025)	9.8±1.35 a (4.89-14.06)
10 days	6	6.0±0.78 b (4-8)	61.5±17.26 a (19-110)	1.3±0.89 b (1-3)	9	57.3±17.40 b (11-129)	6	478.5±112.81 a (258-890)	6.4±0.49 ab (4.48-7.55)
15 days	5	10.6±0.86 a (7-13)	65.6±21.53 a (9-142)	2.4±0.98 b (1-3)	7	73.4±19.73 b (17-167)	5	366.4±123.52 b (65-1098)	3.0±0.77 b (2.10-5.47)
20 days	4	9.0±0.95 a (7-12)	83.5±24.07 a (6-153)	1.3±1.09 b (0-3)	10	56.8±16.51 b (15-183)	4	385.8±138.10 b (51-561)	3.1±0.73 b (1.31-4.87)
Distribution		Gamma	Negative Binomial	Poisson		Poisson		Poisson	Normality (Tukey-Kramer) F=5.08; df=4, 25; P=0.004

^y Within columns, means followed by the same letter do not differ statistically ($P < 0.05$) (all data are mean ± SE).

Table 2. Life table statistics for *Cryptolaemus montrouzieri* kept under different storage periods.

Cohort	n	Intrinsic rate of increase r_m	Net reproductive rate R_0	Mean generation time T_0	Doubling time DT	Finite rate of increment λ
Control	10	0.098±0.003 a	340.703	59.350	7.2	1.101
5 days	7	0.092±0.001 a	276.629	60.851	7.9	1.092
10 days	9	0.074±0.002 b	149.930	67.726	9.3	1.074
15 days	9	0.058±0.002 c	119.321	83.189	12.1	1.059
20 days	11	0.045±0.001 c	65.928	94.173	15.3	1.046

F=47.75; df=4, 41; P=1.10⁻⁵

^y Within columns, means followed by the same letter do not differ statistically (Tukey-Kramer Multiple Comparison Test P<0.05)

mean generation time $T_0 = 40.13$, doubling time $DT = 5.13$, finite rate of increase $l = 1.14$, pre-oviposition period 7.00 days, longevity for males 94.18 and for females 98.08 days. These results are not similar to our control cohort results; dissimilarity may be due to different prey and laboratory conditions.

While the highest intrinsic rate of increase was calculated for the control and 5 day cohorts, the lowest values were obtained for the 15 day and 20 day cohorts. The intrinsic rate of increase value obtained for 10 days cohort was significantly different from those of control cohort and the cohorts kept in different storage periods according to Tukey-Kramer Multiple Comparison Test ($F=47.75$; $df=4, 41$; $P=1.10^{-5}$) (in Table 2). The net reproductive rates dramatically decreased from 340.703 in the control cohort to 65.928 in the 20 day cohort, and decreasing rates ranged 18.81, 55.99, 64.98 and 80.65% for individuals kept in 5, 10, 15 and 20 day storage periods, respectively. Mean generation time increased with increased storage period. The shortest mean generation time value was observed for the control cohort and the value obtained for the 5 day cohort was considerably close to the control cohort. On the other hand, the mean generation time for the 5, 10, 15 and 20 day cohorts was 2.53, 14.11, 40.17 and 58.67% longer, respectively, than for the control cohort. The calculated increased rates of doubling time were very high through the control to the 20 day cohort: 9.72, 29.17, 68.06 and 112.50%, respectively. Population doubling times (DT) for control individuals were 9.72, 29.17, 68.06 and 112.50% longer than for individuals stored 5, 10, 15 and 20 days respectively. When the value of the intrinsic rate of increase was converted into finite rate of increase (λ), the reproductive capacity of the population per female per day for all cohorts decreased from the control to the 20 day cohorts. Finite rate of increase values for all cohorts decreased from the control to the 20 day cohorts. The number of progeny added to a population per female (λ) for the control cohort was 0.82, 2.45, 3.81 and 5.00% higher than in the 5, 10, 15 and 20 day cohorts, respectively.

In Fig. 1, the age-specific mortality was represented as l_x . For the control cohort, l_x curves of females showed a steep slope near the middle of the oviposition period (about 20% mortality), then remained static up to the end of the lifespan. It underwent a very gradual decrease toward the end of the adult stage. The l_x curves showed a typical Type I pattern, as identified by Raymond Pearl, who recognized three survivorship curves²⁶, for the control cohort, which means that most of the mortality occurred late in the adult stage. No significant difference was found between male and female survival rates in the control cohorts, according to the Poisson test and comparison. Survival rates of pairs in the 5 day cohort showed different curves and their differences were significant according to the Poisson test and comparison. The l_x curve of this cohort female showed a gradually decreasing slope. The l_x curve of females showed a Type II pattern, which means that mortality occurred gradually over the lifetime. Survival curves for 10 day, 15 day and 20 day cohorts showed an abrupt drop, especially in the 20 day cohort, and then remained stable up to end of lifetime (lifespan) after the storage periods. The l_x curves for the individuals in these last three cohorts showed a Type III pattern, which means that most of the mortality occurred in the beginning of the adult stage.

The age-specific fecundity was represented as m_x , and the

reproductive value was showed as V_x at x age in Fig. 2. Curves for a unimodal general reproductive pattern, although less irregular, were recorded, and reproductive peaks were observed on the 45^h, 49th and 52nd days in the oviposition period for the control, 5 day and 10 day cohorts, respectively. Although irregular, almost static curves were also recorded for the 15 day and 20 day cohorts in the oviposition period, the pattern was unimodal and showed no conspicuous reproductive peaks. The reproductive value (V_x) per age class of the females in each cohort changed with age and its fluctuations were closely followed by the age specific fecundity curve (m_x) for the control cohort. Reproductive value curves for the 5 and 10 day cohorts followed the fecundity curves in the beginning of the adult stage, but they decreased dramatically due to high mortality rates of females toward the end of their lifetime. Reproductive curves of all cohorts except the 20 day cohort reached a peak at beginning of the oviposition period, and this peak coincided with a maximum peak of age specific fecundity.

Discussion

In this study, we found that long cooling periods greatly affected the mortality and life table parameters of *C. montrouzieri*. Mortality rates increased with increasing cooling periods, especially those longer than 5 days. Results showed that storage periods longer than 5 days are not economical for mass rearing of this predator because of the high mortality rate. Adult longevity was also affected by cooling periods, and the longevity of cohorts exposed to cooling periods was considerably shorter than in the control cohort. The longer the female longevity, the more eggs were laid. According to our results, female fecundity, predator increase rate and prey death rate may increase with longer adult longevity. Predacious coccinellids need to copulate a few times during their life cycle. Özgen and Yasar²⁷ pointed out that different mating numbers affected the net reproductive rate of another coccinellid, *Adalia bipunctata* (L.). We did not separate males and females after emergence. The pre-oviposition period increased with increasing storage period. This was due to the fact that the predator used more energy during cooling periods longer than 10 days, therefore, the energy it needed to mature eggs in the ovary likewise required more time than 10 days. This is not a good characteristic for natural enemies because of the delay in population increase, which would also result in a delayed suppression of the pest. The intrinsic rate of increase (r_m) was the highest for the control and 5 day cohorts. The reasons for the higher intrinsic rate of increase, which is probably the best parameter for comparing treatment effects, were the higher survival rate in first part of the oviposition period, higher daily rate of reproduction and the earlier peak in reproduction. It is well known that even a small reduction in the intrinsic rate of increase can result in great changes in population sizes of pests²⁸.

In general, 5 day storage cohorts were more suitable than the other storage cohorts in terms of adult longevity, reproduction and population growth. According to the results, it is recommend that the cooling period should be 1-5 days at 15°C without prey (food) for storing this predator. Thus, cooling of *C. montrouzieri* serves to both conserve energy for feeding the predator and ensures the availability of adequate food sources, such as potato shoots and vegetable marrow, moreover, adequate predator stock is obtained for critical periods when a large number of predators are needed for release.

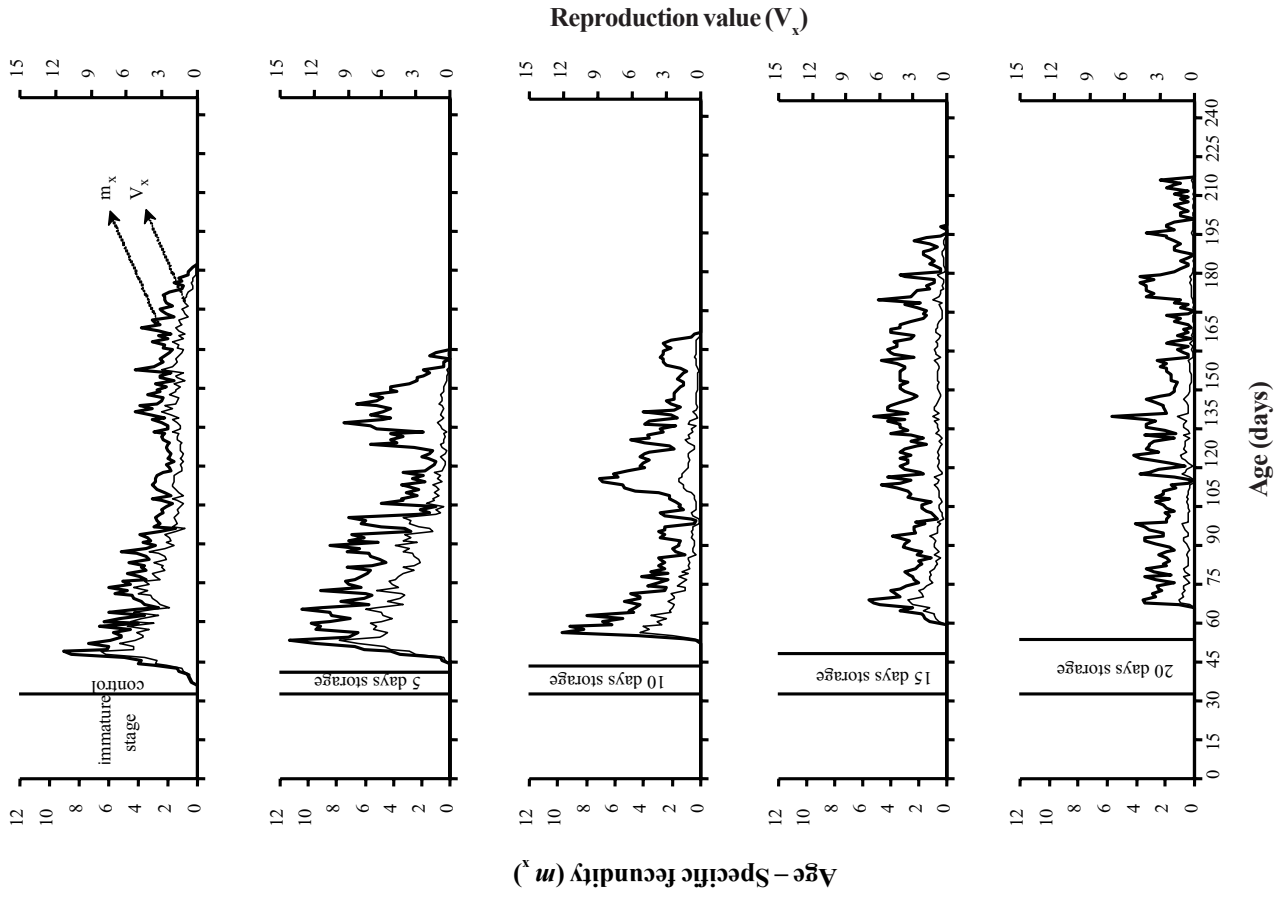


Figure 2. Fecundity and reproduction value curves for *Cryptolaemus montrouzieri* kept under different storage periods.

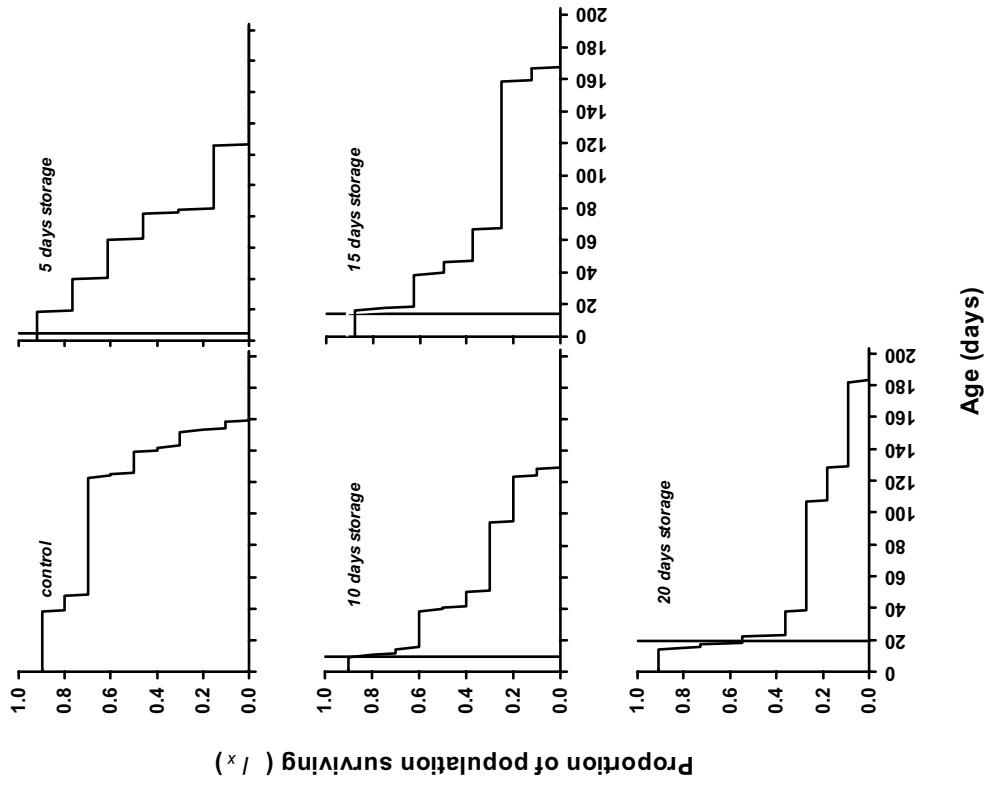


Figure 1. Surviving curves for females of *Cryptolaemus montrouzieri*.

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