# *Improvement of the mass rearing of larvae of the neotropical lacewing* Chrysoperla externa *through the incorporation of a new semiliquid artificial diet*

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# Improvement of the mass rearing of larvae of the neotropical lacewing *Chrysoperla externa* through the incorporation of a new semiliquid artificial diet

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**Abstract** Two new semiliquid artificial diets (A and B), the aphid *Rhopalosiphum padi* L. (Hemiptera: Aphididae) and combinations of these were evaluated to improve the rearing of the Neotropical lacewing predator *Chrysoperla externa* (Neuroptera: Chrysopidae). Additionally, a nutritional analysis of the aforementioned diets was conducted. The developmental time, pupal weight, preoviposition period, fecundity, and fertility were used to evaluate the impact (i.e., potential benefit) of these artificial diets on the predator. We recorded the shortest development time in the treatment group that was given diet A + prey or prey alone ad libitum. Pupal weight was significantly higher with diet A + prey. No difference was found in the female preoviposition period between the five different diets. Fecundity was higher with diet A + prey or prey ad libitum, while fertility on the first day was higher with diet A + prey, cumulative fertility was optimal with diet A and B as food. In conclusion, diet A proved to have the optimal nutritional properties (proteins and carbohydrates) to improve lacewing rearing when used as a nutritional supplement. Furthermore, use of this diet allows a reduction in the use of prey for rearing, and is suitable to be used for mass-rearing programs.

**Keywords** *Chrysoperla externa* (Neuroptera: Chrysopidae) · Biological control · Artificial diets · Mass-rearing programs

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## Introduction

The biological control of pests is one of the most ecologically relevant strategies within the framework cited for integrated pest management and organic crops (van Driesche et al. 2009). Augmentative biological control, where large numbers of natural enemies are periodically released in the crops, is being implemented successfully in several crops worldwide (van Lenteren 2000; van Lenteren and Bueno 2003). As of now, more than 125 species of natural enemies are on the market for biological control of pests (van Lenteren 2003). The production of these organisms—

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mostly predators and parasitoids—should be cost-effective, requires adequate nutrients to be provided through the diet, and requires an adequate reproductive capacity (Cohen 2004).

Nowadays, the production of predators for biocontrol is commonly based on the use of factitious prey, i.e., the eggs of *Sitotroga cerealella* (Lepidoptera: Tineidae) or *Ephestia* (*Anagasta*) *kuehniella* (Lepidoptera: Pyralidae) for *Chrysoperla carnea* (Neuroptera: Chrysopidae), *Macrolophus pygmaeus* (Heteroptera: Miridae), *Orius laevigatus* (Hemiptera: Anthocoridae) among others (Bonte and De Clercq 2008; Vandekerkhove et al. 2008), involving high costs in the mass rearing of natural enemies for field-releasing (the market price of the factitious prey eggs of *E. kuehniella* is around 650 \$US $kg^{-1}$, Vandekerkhove et al. 2008). In other instances, biocontrol producers need to rear the prey of the natural enemy and the corresponding host plant in a tritrophic system. This approach entails high labor costs for the operation and maintenance of separate spaces and the appropriate equipment to produce each trophic level (Riddick 2009). In contrast, the use of an artificial diet allows a reduction in or elimination of plant materials and prey colonies in the rearing system, leading to lower production costs (Bonte and De Clercq 2010).

The subsistence of healthy laboratory insects depends on the quality of their diets, which must provide nutritional support for basic metabolism, development, and reproduction. The nutritional needs vary with the stage and the physiologic state and are generally higher for larvae than for adults (Grenier 2012). Within this framework, a completely artificial diet must contain a nitrogen source, lipids, carbohydrates, vitamins, and minerals in addition to stabilizers and preservatives. Proteins are used as the principal source of nitrogen by most insects, and after the degradation of those proteins to amino acids the latter are reutilized for the synthesis of the insect-specific proteins of muscles, enzymes, and certain hormones and for structural elements. Likewise, lipids are essential for the structure of cell membranes, hormones, nutrient transporters, and sources of energy and as raw materials for building other molecules. Finally, carbohydrates function as receptors and channels for the movement of materials into and out of the cells and furthermore serve not only as structural elements and energy sources but also as constituents of glycoproteins (Cohen 2004).

The nutritional plasticity of certain predator species—as demonstrated by the consumption of non prey foods such as nectar, honeydew, and pollen—is a positive characteristic that makes them more easily reared on an artificial diet (Specty et al. 2003). Those alternative natural components increase the survival when the prey is scarce, reduce the mortality during diapause, and enhance the reproductive capacity of the insect (Lundgren 2009). This same plasticity is also modulated by the reproductive strategy of the species. One example is the occurrence of proovigenic females whose eggs are already mature before adult emergence (Jervis et al. 2007), whereas with the alternative strategy, referred to as synovigeny, the egg complement is formed after emergence of the adult (Jervis and Ferns 2005). Intermediate states exist between these two strategies. Therefore, in a proovigenic species, larval nutrition during mass rearing is critical since the fat bodies of the larval stages are responsible for vitellogenesis, i.e., the materials used for egg maturation in the imago derive mostly or entirely from previously stored larval resources.

The Chrysopidae—among the most extensive families of neuropterans and the most valuable ones in economic terms (Senior and McEwen 2001)—consists of a large group of insects with a wide geographic distribution that feeds on a diversity of prey such as aphids, coccids, and other soft-bodied pests, thus diminishing their populations in agroecosystems below the economic-injury level (Tauber et al. 2000). For this reason, several species of *Chrysoperla* Steinmann have received special attention as biological control agents of pests (Díaz-Aranda and Montserrat 1995) and are all produced for commercial use by feeding on a factitious prey such as *S. cerealella* (Albuquerque et al. 1994; Legaspi et al. 1994; Soto and Iannacone 2008) or *E. kuehniella* eggs (Boregas et al. 2003; Cohen and Smith 1998; Hilbeck et al. 1998). Nevertheless, this type of rearing could make predator production too expensive for large-scale use and open-field agricultural settings. Considerable progress has been made in the manipulation of *Chrysoperla* spp. colonies for biological control programs through the development of several artificial diets. None of these, however, have been incorporated into a large-scale production system, and different explanations have been given for this lack of success, e.g., the complexity of manufacturing and production expense of the diet along with the higher

performance of *Chrysoperla* reared on the natural or factitious prey compared with diet-reared insects (Cohen and Smith 1998).

*Chrysoperla externa* Hagen is widely distributed from the southeastern United States and the Antilles to southern South America (Albuquerque et al. 1994). The species has been considered as an excellent potential biological control agent because of its high adaptive capacity to different ecosystems and a promiscuous feeding ability, involving a broad range of prey (Albuquerque et al. 1994). In Argentina, *C. externa*—located as far south as northern Patagonia—is associated with crops, fruit trees, and grasses adjacent to cultivated land (González et al. 2011). In the Buenos Aires province, the species has been recorded as a relevant predator in the protection of extensive (i.e., soybean) and intensive (i.e., sweet pepper) crops (Haramboure et al. 2014; Rimoldi et al. 2008). The three larval instars exhibit predatory behavior, thus being effective natural enemies of mites and insects such as coccids, aphids and eggs of lepidopterans and thrips (Bastidas et al. 2010; Soto and Iannacone 2008). *C. externa* can easily be reared in the laboratory, thus enabling the development of improved techniques for its production in view of the demands for rearing and maintenance exacted within the context of research or commercialization (Boregas et al. 2003).

The aims of this research were: (1) to evaluate the nutritional quality of two new semiliquid artificial diets for the development and reproduction of *C. externa* over the species' different biological stages compared with that on different densities of prey, and (2) to analyze the biochemical composition of the diets in relation to their nutritional value in supporting reproductive performance. Finally, we discuss the potential use of these semiliquid artificial diets in the mass rearing of this commercially significant predator.

## Materials and methods

### Insects and plant materials

Adults of *C. externa* were collected from vegetable crops within the environs of La Plata (34° 59′ 10.06″S; 57° 59′ 52.77″W), Argentina. In the laboratory, the adults were conditioned in ventilated plastic containers (15 cm diameter, 9 cm high) covered with a fine mesh and quarantined to minimize parasitism and disease prior to rearing. Once quality and health of collected material were corroborated in quarantine, their progeny was used to initiate the laboratory colony of the species. Chrysopid adults, whose diet is not prey but based on pollen and nectar, were reared on an artificial diet based on honey, wheat germ, and brewer's yeast, which is commonly used for adults of the species (Vogt et al. 2000). The colony reproduced for several generations, and the wild stock (between 50 and 80 adults), collected yearly from the same geographical origin, was crossbred to help maintain its genetic variability.

The bird-cherry aphid, *Rhopalosiphum padi* L. (Hemiptera: Aphididae), was used as prey. An aphid colony was initiated from clones obtained from the School of Agricultural and Forestry Sciences (National University of La Plata, Argentina) and was reared on pesticide-free wheat seedlings (*Triticum aestivum* L., cultivar ACA 901). These seeds were germinated in plastic pots (6 cm high, and 6 cm in diameter) with standard substrate (fertile soil and perlite 1:1 [v/v]) and infested with aphids at germination. The seedlings were maintained in ventilated plastic boxes (13 cm high, × 13 cm long, × 23 cm wide). The insect colonies and all bioassays were carried out in a growth chamber with controlled environmental conditions (23 ± 5 °C, 70 ± 5 % RH, and a 16:8-h light:dark period).

### Composition analysis and cost of artificial diets

Two new semiliquid artificial diets were designed and developed under aseptic conditions in our laboratory (Schneider, unpublished data), herein after referred to as diet A and diet B (Table 1). Both diets were supplied to larvae in spherical packages which were prepared by placing approximately 0.2 g of the diet on a 2 × 2 cm square of Parafilm® and then joining the square corners together. These spheres were supplied once every two days. The ingredients of both diets were collected and afterwards the costs for making 1 kg for each diet were calculated to compare with the cost of predator rearing with factitious prey.

The two artificial diets were analyzed to determine their nutritional content in terms of the percent composition of carbohydrates, proteins, and lipids. Two samples per diet were sent to the Center of Research and Development in Food Cryotechnology,

**Table 1** Ingredients of the semiliquid artificial diets A and B. Amounts are per 100 gr of diet

| Ingredients | Diet A | Diet B |
|---|---|---|
| Liver | 16 gr | 10 gr |
| Ground beef | 16 gr | 10 gr |
| Fructose | 8 gr | 10 gr |
| Glucose | 8 gr | 10 gr |
| Sucrose | 8 gr | 10 gr |
| Honey | 6 gr | 10 gr |
| Condensed milk | 3 gr | – |
| Wheat germ | 8 gr | 5 gr |
| Brewer's yeast | 5 gr | 3 gr |
| Egg | 8 gr | 30 gr |
| Egg white | 11 gr | – |
| Multivitamin* | 0,5 gr | 1 gr |
| Formaldehyde | 1 ml | 0.15 ml |
| Sodium Benzoate | 0.5 gr | 0.1 gr |
| Nipagin** | 0.1 gr | 0.3 gr |

* Multivitamin Centrum®: A mixture containing vitamin A (2.000 IU), ß-carotene (2.000 IU), vitamin D3 (200 IU), vitamin E (14.9 IU), vitamin K1 (30 µg), vitamin C (60 mg), folicacid (200 µg), vitamin B1 (1.4 mg), vitamin B2 (riboflavine; 1.6 mg), niacinamide (18 mg), vitamin B6 (2 mg), vitamin B12 (1 µg), pantothenicacid (6 mg), biotin (150 µg), calcium (162 mg), phosphorus (125 mg), iodine (150 µg), iron (14 mg), magnesium (100 mg), copper (2 mg), zinc (15 mg), manganese (2.5 mg), potassium (40 mg), choride (36.3 mg), chromium (25 µg), molybdenum (25 µg), selenium (25 µg), nickel (5 µg), tin (10 µg), silicon (2 µg), vanadium (10 µg)

** Methyl 4-hydroxybenzoate: an antifungal agent

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The Ratzlaff method, i.e., an acid treatment followed by extraction with an organic solvent, was used to determine the lipid content after cleaving the protein-lipid bonds in the sample. Proteins were extracted by the Kjeldahl method (conversion factor, 6.25) and carbohydrates by the Fehling-Causse Bonnans procedure.

### Development and reproduction bioassays

Cohorts of neonates of *C. externa* (24 h after hatching) were randomly selected from the predator colony and used for the bioassays. The larvae were placed in ventilated Petri dishes with a fine brush. Five treatments involving different nutritional regimens were carried out: (a) diet A, (b) diet B, (c) adults of *R. padi* at a low prey density (20 aphids per larva), d) adults of *R. padi* ad libitum (50, 100, and 150 aphids applied to the 1st, 2nd, and 3rd larval instars, respectively), and finally e) a combination of diet A and prey at low density (20 aphids per larva). The combination of diet A + prey was chosen over diet B for this experimental condition based on pilot experiments (Haramboure M, unpublished data) where diet A had shown more favorable results than diet B. Each Petri dish was checked every two days for replenishment of the corresponding food. The nutritional quality of all diets was assessed through the following biological parameters: The developmental time of the larval and pupal stages was checked daily and recorded until the emergence of adults. In addition, the pupal weight was measured on an analytical balance (Acculab®) at a precision of $10^{-4}$ g. Once emerged, adults were identified as females or males by the external genitalia and then paired. The preoviposition period (from adult emergence to the first day of oviposition), fecundity (number of eggs laid per day), and fertility (number of hatched larvae per day) were observed daily. The last two parameters were estimated as the total over a period of five days.

### Statistical analysis

Data were tested by the analysis of variance (ANOVA) after being log-transformed [log (x + 1)] when this was necessary, to insure normality and homoscedasticity of variances, or Kruskal–Wallis test when ANOVA assumptions were not met. Multiple comparisons between means or medians were done with a Fisher's least-significant-difference or Box and Whisker plot tests, respectively and $P < 0.05$ was considered significant (Scheiner and Gurevitch 2001). These analyses were performed using the Statgraphic v. XVI software (STSC 2013).

## Results

### Composition analysis and costs of diets A and B

An analysis of the dietary macrocomponents indicated specific differences between the basic composition of the two diets A and B (Table 2): Diet A contained 2 %

**Table 2** Percent composition of the semiliquid artificial diets (A and B). Data are means ± SE

| Diet | Water | Proteins | Lipids | Carbohydrates | Nonhydrolyzed carbohydrates | Ashes |
|---|---|---|---|---|---|---|
| A | 51.05 ± 0.05 | 12.35 ± 0.05 | 3.25 ± 0.05 | 18.05 ± 0.21 | 13.3 ± 0.34 | 1 1.98 ± 0.05 |
| B | 59.3 ± 0.9 | 10.15 ± 0.05 | 7.22 ± 0.11 | 20.50 ± 0.60 | 1.2 ± 0.04 | 1.59 ± 0.01 |

more proteins than diet B, whereas diet B, contained more than twice the lipids than diet A. Conversely, the hydrolyzed carbohydrates of diet B were a little over 2 % more than in diet A, but the nonhydrolyzed-carbohydrate content of the latter was over ten times that of diet B. On the basis of these macrocomponents only, we determined the caloric value of the two diets to be about 150 kcal per 100 g for diet A and 188 kcal per 100 g for diet B. For the rearing of one thousand individuals, the cost of diet A and B was around 20 and 40 \$US $kg^{-1}$, respectively.

### Development time

The development time of each larval instar of *C. externa* varied with the different treatments (Table 3). However, we observed no differences between diet A + prey and prey ad libitum: the predator reached the pupal stage the earliest when the larvae were fed on diet A + prey and with prey ad libitum. Diet A alone or a low prey density resulted in a significantly longer developmental time than diet A in combination with the prey, while diet B resulted in the latest pupal formation of all treatments.

### Pupal weight

Pupal weights differed among the four diets (Fig. 1). The largest pupae resulted from feeding on diet A + prey, followed by prey ad libitum and diet A alone. Diet B and prey at low density gave rise to significantly smaller pupae.

### Preoviposition period, fecundity and fertility

The preoviposition period was not affected by the different larval diets (Table 4): the time between the emergence of the adult females and their subsequent oviposition was five days under all dietary treatments. However, we noted significant differences in the adult-female fecundities on the first day of oviposition after having been fed on the different diets as larvae (Fig. 2). Females from the larvae fed on prey ad libitum exhibited a significantly lower fecundity on the first day, whereas the highest oviposition rate was observed with diet A + prey. Cumulative number of hatched eggs was lowest on diet B, and highest with prey ad libitum or diet A + prey.

The percentage of eggs hatched on day 1 (Fig. 3) was low when the larvae had been fed on a low prey density or diet B. The percentage increased significantly after having fed on diet A + prey, whereas feeding on diet A or prey ad libitum resulted in intermediate hatching percentages. The cumulative fertility followed a different pattern in relation to the cumulative fecundity with a significantly higher number of hatched eggs with diets A and B, intermediate values for diet A plus prey or prey ad libitum, and the lowest number with low prey density as food.

## Discussion

In recent years, the rearing of the natural enemies (predators and parasitoids) of pests in the laboratory has been of pressing, albeit limiting, concern in the mass production of those biological control agents, along with their implementation in the framework of integrated pest management (IPM) programs. Because the main problem of mass rearing of predators is the high economical costs of natural or factitious prey, the aim of our work was to evaluate a low-cost artificial diet with all the required nutritional properties. For the feeding of one thousand larvae, for example, we would need 2 kg of diet A alone (35 \$US), 3.2 kg of diet B alone (112 \$US), or 1.2 kg of diet A (21 \$US) if we combine it with prey at low density (taking into account the differences in development time with each regime. See Table 3). These values are much lower than those reported by Vandekerkhove et al. (2008) when commercialized factitious prey were used for mass rearing of several predators (650 \$US $kg^{-1}$).

**Table 3** Immature-development time of *Chrysoperla externa* reared under different diets. L1, first instar; L2, second instar; L3, third instar; P, pupa; A, adult

| Diet | Development time (days) | | | | |
|---|---|---|---|---|---|
| | L1–L2[a] | L2–L3[b] | L3-P[c] | P-A[d] | L-P[e] |
| A | 7.357 ± 0.179g | 6.125 ± 0.125g | 4.142 ± 0.142f | 12.25 ± 0.137h | 19.2 ± 0.513g |
| B | 11.315 ± 0.382h | 10.031 ± 0.696h | 10.625 ± 0.727h | 13.428 ± 0.202i | 33.027 ± 1.431h |
| A + prey | 5.522 ± 0.106f | 3.0 ± 0.186f | 4.08 ± 0.114f | 11.478 ± 0.106g | 12.538 ± 0.159f |
| Low prey density | 5.351 ± 0.401f | 6.2 ± 0.255g | 6.205 ± 0.256g | 10.724 ± 0.284f | 17.529 ± 0.254g |
| Prey ad libitum | 5.428 ± 0.254f | 3.421 ± 0.206f | 4.523 ± 0.202f | 10.84 ± 0.179f | 14.095 ± 0.275f |

Data are mean ± SE. Within each column, different letters denote significant differences between diets (Kruskal–Wallis and Box and Whisker Plot tests, $P < 0.05$)

[a] $H = 99.069$; $df = 4$; $P < 0.001$

[b] $H = 75.135$; $df = 4$; $P < 0.001$

[c] $H = 84.316$; $df = 4$; $P < 0.001$

[d] $H = 66.763$; $df = 4$; $P < 0.001$

[e] $H = 129.028$; $df = 4$; $P < 0.001$

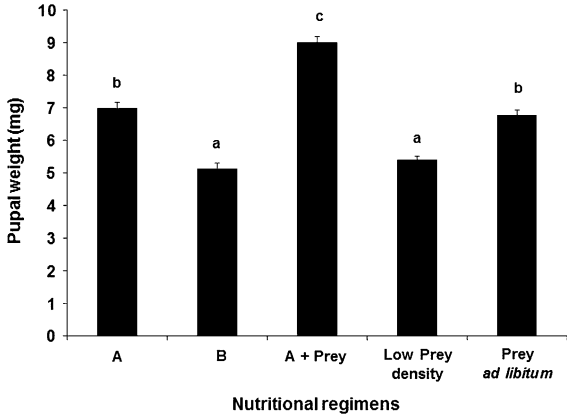


**Fig. 1** Pupal weight of *Chrysoperla externa* from larvae reared on different diets. The data are the mean + SE. Different letters denote significant differences between diets (ANOVA, Fisher LSD, $P < 0.05$) ($F = 93.69$; $df = 4, 138$; $P < 0.001$)

**Table 4** Preoviposition period of *Chrysoperla externa* females fed with different diets

| Diet | Preoviposition period (days) |
|---|---|
| A | 5.4285 ± 0.202a |
| B | 6.4285 ± 0.812a |
| A + prey | 6.2500 ± 0.491a |
| Low prey density | 5.5000 ± 0.377a |
| Prey ad libitum | 5.1818 ± 0.263a |

Data are mean ± SE. Different letters denote significant differences between treatments (Kruskal–Wallis, Box and Whisker Plot tests, $P < 0.05$)

$H = 3.96$; $df = 4$; $P = 0.41$

According to our calculations, these artificial diets are about 85 % cheaper than the use of factitious prey.

In the present experiments, the packaging of the diet into small spheres wrapped in stretched Parafilm® was done to resemble the size and shape of the natural prey. All Neuropteran larvae, including chrysopids, have suctorial mouthparts that consist in feeding tubes formed by the mandibles and maxillae (Monserrat et al. 2001). Since the chrysopid larvae attack their prey by inserting their jaws and injecting salivary secretions to liquefy the internal tissues through extraoral digestion (Syed et al. 2008), the Parafilm® mimicked the prey's cuticle, needing to be perforated to extract the contents. This type of penetration and digestion allowed the larvae to attack larger prey, where a spherical body of double or triple the size of a larva is not only accessible but also becomes more suitable than small pieces of food material (Grenier 2012). This mode of feeding dictates the acceptability of artificial diets by predators (Cohen and Staten 1993; Grenier et al. 1994; De Clercq et al. 1998; Cohen 2004).

The larval development time of these insect predators is affected by their diet (Osman and Selman 1996, Principi and Canard 1984), and if the aminoacids necessary for larval growth are not acquired during the immature stages, the subsequent stages would be

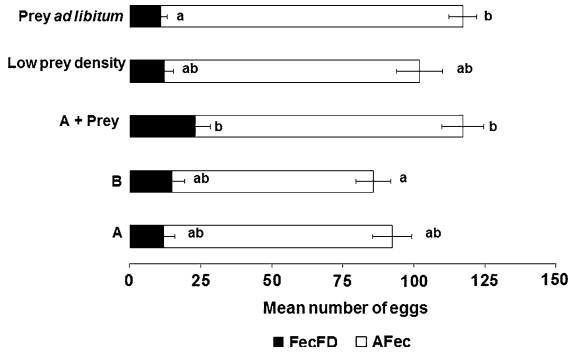


**Fig. 2** Fecundity of *Chrysoperla externa* females from larvae reared on different diets. Fecundity on the first day (FecFD: black part of the bars) and cumulative fecundity over the first five days (AFec: entire bar length). The data are the mean ± SE. Different letters denote significant differences between nutritional treatments (ANOVA, Fisher LSD, $P < 0.05$) (FecFD: $F = 3.39$; $df = 4, 36$; $P = 0.019$; AFec: $F = 3.04$; $df = 4, 36$; $P = 0.029$)

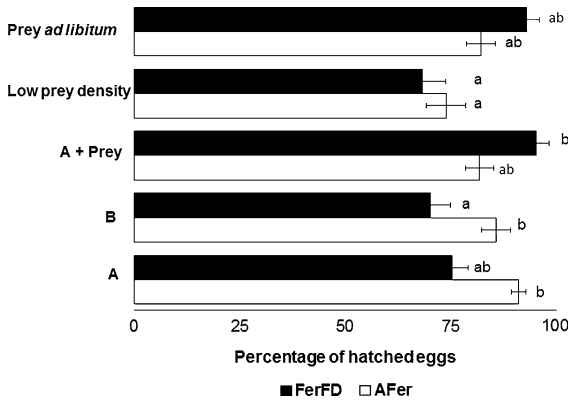


**Fig. 3** Fertility of *Chrysoperla externa* females from larvae reared on different diets. Fertility on the first day (FerFD; *black bars*) and cumulative fertility over the first five days (AFer; *white bars*). The data are the mean ± SE. Different letters denote significant differences between nutritional treatments (ANOVA, Fisher LSD, $P < 0.05$) (FerFD: $F = 3.39$; $df = 4,36$; $P = 0.018$; AFer: $F = 2.71$; $df = 4, 36$; $P = 0.045$)

affected, increasing the total duration of the life cycle (Scriber and Slansky 1981; Thompson 1999; Stathas 2001). In our studies, the immature development time of *C. externa* was shortened when the larvae were fed on diet A + prey, which could be related to the higher protein content of this diet.

The development time of the immature stages (*cf.* Table 3) was shorter with prey ad libitum or diet A + prey than with the three remaining diets. Other studies have demonstrated that an artificial diet alone was not as suitable for predators as natural prey because of the presence of specific nutrients in the latter (Riddick 2009; Silva et al. 2009). Moreover, those authors observed that a diet of high prey density was comparable to one of low prey density. In contrast, and along with the present results, Atlihan et al. (2004) noted that individuals of *C. carnea* completed their immature stages sooner with increased prey numbers. Our results, furthermore, demonstrate that a low prey density supplemented with diet A had the same effect on the developmental time as did feeding on the prey ad libitum. Nevertheless, the presence of the prey as diet is crucial for the development of first instar. For this reason, the diets A or B alone were not of sufficient nourishment for this instar.

From the order of the pupal weights registered on the five diets (Fig. 1)—diet A + prey > diet A or prey ad libitum > diet B or low prey density—diminished prey densities did not result in optimal development of the larvae. Since diet B apparently contains insufficient protein, it might have been rejected by larvae, thus resulting in a low weight at the pupal stage. Diet A + prey resulted in much larger pupae. Their weight was similar to that obtained by Syed et al. (2008) for *C. carnea* fed on different nutritional regimens.

The preoviposition period, i.e., the time between the emergence of the adults and the first oviposition, did not differ with feeding on the five diets. During the period between emergence of adults and first oviposition, the pre-oviposition females could have fed on the adult artificial diet, which could have resulted in the uptake of nutrients lacking during their immature stages. In contrast, the females of *C. carnea* exhibited a shortened preoviposition period on a diet with an increased prey density (Atlihan et al. 2004), while Jokar and Zarabi (2014) registered a longer preoviposition period of *C. carnea* when those larvae were fed an artificial diet alone, and no differences on a diet consisting of a combination of *S. cerealella* eggs + an artificial diet or on a diet of the *S. cerealella* eggs alone.

The large-scale production of predatory insects for augmentative biological control is frequently restricted by a suboptimal fecundity of predators reared on artificial diets (Grenier 1994). We therefore analyzed the fecundities and fertilities on the first day and those accumulated over the first five days. These

parameters, critical for the performance of *C. externa* as a biological control agent, are linked to oogenesis since fecundity is strongly influenced by imaginal feeding in proovigenic insects such as chrysopids (Canard and Volkovich 2001).

When larvae fed on prey ad libitum, the low oviposition rate on the first day (Fig. 2) indicated that this natural diet did not insure a high fecundity at this time. In contrast, the highest number of eggs initially laid was after feeding with diet A + prey, implying that rearing on diet A improved the reproductive development of the females. The same was observed for the cumulative fecundity, where feeding with diet A + prey resulted in a significantly higher oviposition rate than with the other diets. The higher percentage of protein in diet A compared to diet B could explain this observed difference in fecundity. Indeed, reduced egg production has previously been observed in different experiments on predatory insects reared on artificial diets, where the decreased fecundity has been attributed to a deficiency in proteins that led to a lack of mature-follicle formation (Adams 2000; Ferkovich and Shapiro 2004).

Larval feeding on diet A + prey likewise produced the highest number of larvae hatched on the first day of oviposition (Fig. 3). Based on the combined fecundity and fertility results, the total number of offspring per female was higher when larvae were reared on diet A + prey. Jokar and Zarabi (2014) reported maximum fecundity values for *C. carnea* females when a combination of an artificial diet and *S. cerealella* eggs had been supplied to *C. carnea* larvae, but the highest percentage of hatched eggs was found when the larvae had been fed *S. cerealella* eggs alone.

In the present experiments, however, the cumulative fertility showed a different pattern: although lower values were obtained with prey at low density, the rest of the dietary regimens resulted in higher values (70–80 %). As alluded to above, this difference could be explained considering that adults were provided with a different type of artificial diet so that during the first five days of adult life, the reproduction parameters were affected by this regimen in a manner superimposed upon the original nutritional effects of the diets supplied during those insects' immature development.

In conclusion, the results obtained in this study indicate that the low-cost artificial semiliquid diet A is an excellent complement to an *R. padi*–based diet. This combination may prove to be an optimal diet for mass-rearing of *C. externa* in biological-control programs. The most important characteristics of a natural enemy for any mass-rearing program are a fast developmental rate and a high rate of reproduction (Hajek 2004; Qiu et al. 2004). These are the parameters that where highest on a combination of diet A and natural prey.

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