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How much does a drop of sugar solution benefit a hatchling of *Chrysoperla pallida* (Neuroptera Chrysopidae)?

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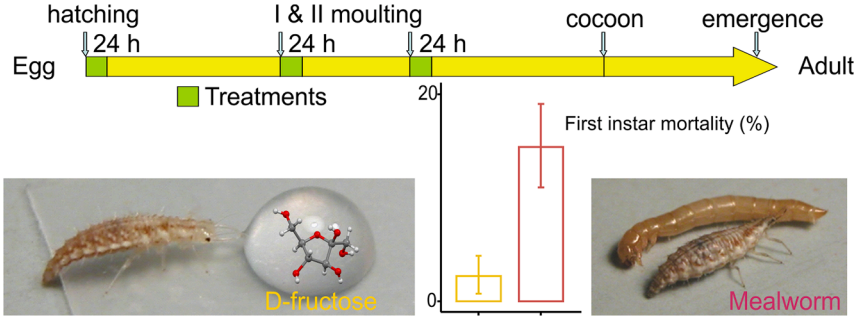
HIGHLIGHTS

- Non-prey foods are an integral component of the diets of predaceous chrysopid larvae.
- Chrysopid larvae benefit from sugary food especially after hatching.
- Hatchlings were less likely to die feeding on sugar solution alone than on prey alone.
- Fastest growth occurred when hatchlings had access to both prey and sugar solution.

GRAPHICAL ABSTRACT



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ABSTRACT

Although primarily predaceous, chrysopid larvae are well known to also use honeydew, floral and extrafloral nectar, and other plant-based sugary nutrients as food. However, the extent to which the three larval stages ingest sugary liquids and the value of this sugar-feeding to subsequent survival and development (if any) have not been quantified. Here, our first experiment examines how much sugary liquid is ingested when it is offered to newly hatched or newly moulted larvae of *Chrysoperla pallida*. After the intake of a fructose solution, the average weight of hatchlings was almost tripled and that of freshly moulted larvae was increased by 57% (II instar) and 26% (III instar). The second experiment was designed to identify and examine the effects of larval ingestion of fructose liquid on subsequent development and survival. In this experiment, each larva was subjected to a 24-hour period of a dietary treatment three times during its development: once soon after hatching and twice again, soon after each of the two larval moults. The dietary treatments were: 1) no provision – without food or water; 2) water only; 3) fructose solution only; 4) mealworm only; 5) mealworm, with water; 6) mealworm, with fructose solution. During the periods between treatments, the larvae were fed mealworms. This experiment

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demonstrated that access to a sugar solution had significant positive effects on larval performance. Larvae with the fructose solution alone were significantly less likely to die than those with no provisions, water only, or, surprisingly, mealworm alone. Moreover, fastest development occurred when larvae had access to both mealworm and the fructose solution during the first 24 h of each instar. Most of the mortality occurred during the first instar, and we did not detect any effects of the treatments on the cocoon stage, neither on mortality within the cocoon nor on weight. We conclude that sugar alone does not lead to increased biomass; rather, it promotes longevity and together with a protein source, it enhances the growth rate compared to a diet of protein only. This demonstration of significant positive life-history effects from sugar intake, especially for first instars, has valuable practical application: first, for mass rearing and lab rearing methods, and second, in the context of conservation biological control.

1. Introduction

Most predaceous terrestrial arthropods are known to also feed on honeydew, floral and extrafloral nectar, and other plant-provided food (Polis and Strong, 1996; Coll and Guershon, 2002; Wäckers et al., 2005; Lundgren, 2009). Chrysopids are no exception, and this statement is true not only for the phytophagous adults of most genera, but also for the carnivorous larvae and adults.

Sugar feeding among chrysopid larvae has been well known and documented for a long time. Smith (1922) appears to have been the first to report that larvae feed on "drops of plant juice" in the field and sugar solution in the laboratory. Rabaud (1926) described how larvae of *Chrysoperla* feed regularly on floral nectaries in the field, and later zoophytophagy by chrysopid larvae was reported and studied by many others (e.g. Kawecki, 1932; Killington, 1928, 1936; Principi, 1940; Downes, 1974; Keeler, 1978). The opinions of authors regarding the role of sugar feeding in larval survival and development have varied from it being unlikely that plant juices formed an important part of the larval diet (Killington, 1936; Principi, 1940; Principi and Canard, 1984) to assuming that sugar solutions (carbohydrates) are almost sufficient for larval development (Rabaud, 1926), or that they form a normal part of an otherwise largely carnivorous larval diet (Downes, 1974).

Chrysopids, mostly those belonging to the genus *Chrysoperla*, are very effective biological control agents, and much effort has been devoted to developing methods for mass-producing them in large numbers for mass release into agricultural system (Tauber et al., 2000; Senior and McEwen, 2001). Research in this area, especially the pioneering studies by K. S. Hagen and colleagues, provided significant information on chrysopid nutritional requirements in general; they demonstrated the value of including sugar in the artificial larval diets of chrysopids (Hagen and Tassan, 1965; Hagen, 1987). Finally, McEwen et al. (1993) demonstrated that larvae provided with drops of artificial honeydew were "more likely to complete their development and did so significantly more rapidly" compared to larvae not fed honeydew during mass rearing.

More recently, in line with the growing interest in plant-provided food for predaceous insects, the pivotal paper of Limburg and Rosenheim (2001; see also the preliminary note: 1998) provided the first, and until today, the only quantitative reports on field nectar consumption by chrysopid larvae. Their results are consistent with the hypothesis that nectar feeding may support longevity and/or prolong foraging activity. Later, Patt et al. (2003), using stable isotope analysis, demonstrated, on the one hand, that pollen and artificial nectar complement the nutrients obtained from suboptimal prey, and on the other, that larvae acquire a significant amount of carbon from sucrose in a mixed prey/non-prey diet. Lastly, Hogervorst et al. (2008) showed that chrysopid larvae "… use honeydew as a food source in the presence of suitable prey".

Today, sugar solutions (nectar and honeydew in the wild, diluted honey or solute sugar in the laboratory) are considered a fundamental component of chrysopid larval nutritional ecology. Larval performance can be enhanced when larvae have mixed diets compared to prey-only. The energy content of 'sweet' foods is often similar to that obtainable from prey. Moreover, sugar feeding can provide a means for chrysopid larvae to bridge periods of low prey availability. However, it has remained unknown how much and when, during larval development, a sugar supplement benefits larval performance.

In the study reported here, we pose the hypothesis, derived from earlier observations in our laboratory, that chrysopid larvae benefit from a sugar supplement not only during periods of prey scarcity, but regularly during normal larval development. To being evaluating this hypothesis, we conducted two experiments. The first examined if and how much sugar solution newly hatched and recently moulted lacewing larvae ingest when the solution is offered under experimental conditions. The second experiment examined which components of larval performance were affected when diets with or without fructose were presented at specific times during development. These components included: larval survival rates, first instar mortality, larval developmental time, and subsequent larval-pupal mortality and weight within the cocoon.

2. Materials and methods

2.1. Experimental animals and experimental conditions

2.1.1. Lacewing

Our study focused on the green lacewing species *Chrysoperla pallida* Henry, Brooks, Duelli, Johnson, 2002. The species was identified by visual analysis of its species-specific courtship call (Pantaleoni and Sechi, 2014). Because the abdominal vibrations act on a single plane, the call signal can easily be transduced into a visual signal useful for identification.

For our experiments, we used the F_2 offspring of adult females collected in the field, from Sassari (Northern Sardinia, Italy), and then reared in the laboratory, under conditions of 25 ± 1 °C temperature, 65 ± 5% relative humidity, and 16:8 light/darkness. The adult lacewings received a diet composed of bee pollen and water. As factitious prey, their larvae were provided mealworms (larvae of *Tenebrio molitor* Linnaeus, 1758, Coleoptera Tenebrionidae) (Loru et al., 2014).

2.1.2. Factitious prey

Mealworms (larvae of *Tenebrio molitor*) from a twenty-year-old insect culture at the Italian National Research Council (CNR) in Sassari were reared on bran flour, mixed with a blend of fresh vegetables, under conditions of 25 ± 1 °C temperature, 65 ± 5% relative humidity, and 16:8 light/darkness (Cotton, 1940). Following Loru et al. (2014), the size of mealworms used as prey was proportionate to the instar of the predator: 4–5 mm long and 0.5–1 mg in weight for I instar, 6–8 mm long and 2–4 mg in weight for II instar, 9–12 mm long and 6–12 mg for III instar. Prior to use as prey, the mealworms were killed with ethyl acetate, a routine and safe procedure used to make the factitious prey harmless and readily available.

2.1.3. D-fructose water solution

The sugar solution used in both experiments was a 10% (weight/weight) solution of D(–)fructose [98%] in oligomineral water [Smeraldina®, from springs in Monti di Deu, Tempio Pausania (Northern Sardinia)]. In our tests, sugar solution drops of 2, 5 and 10 μl were used for hatchlings, freshly moulted II instar, and freshly moulted III instar,

respectively.

2.1.4. Experimental arena

The experimental arena for both experiments was a transparent polystyrene cylindrical container with pressure cap 25/25 mm height/diameter. A square of paper and a smaller square of Parafilm® was placed on the bottom of the container and the diet was then placed on the Parafilm®.

2.2. Experimental design

2.2.1. First Experiment: Ingestion of sugar solution

A total of 22 II and 18 III instars, all freshly moulted and ramdomly selected from the culture, were weighed before and after being kept in the experimental arena with the sugar solution for 24 h. The difference in weight before and after the treatments indicated how much sugar solution was ingested.

The weight of the I instars was close to the sensitivity limit (fine range 10 μg) of the analytical scales (Mettler Toledo AX 105 DeltaRange). Thus, for each first instar we repeatedly weighed each individual 10 times. To prevent bias related to the handling of this very small and delicate instar, the 10 larvae that were randomly selected and weighed before feeding were not the same as the 10 larvae that were randomly selected and weighed afterwards.

2.2.2. Second Experiment: Survival and developmental responses to diets with and without fructose

This experiment had six treatments, with one hundred larvae for each one. The treatments were:

(1) no provision (i.e., without fructose solution, water, or mealworm).
(2) water only.
(3) fructose solution only.
(4) mealworm only.
(5) mealworm, with water.
(6) mealworm, with fructose solution.

The test was carried out in the arenas described above. Each experimental larva was subjected to its treatment for 24 h three times during its development: once soon after hatching and for the second and third times soon after each of the two larval moults, respectively. The size of the drops of water or fructose solution and the size of mealworms increased with size of the instar (see above). During the periods between treatments, the larvae were fed, on alternate days, with two mealworms. All the larvae were regularly checked every 24 h. Ecdysis, cocoon spinning, and any dead larvae were recorded. Ten days after spinning, cocoons containing the pupa were weighed (= "cocoon weight"). Subsequently, after emergence, the sex of each adult was registered.

2.3. Analysis of results

2.3.1. Survival

We analyzed survival in the larval stage from hatching to cocoon spinning using a Cox proportional hazard regression model, with the experimental treatment group ('1 no provision'; '2 water only; '3 fructose solution only'; '4 mealworm only'; '5 mealworm, with water'; '6 mealworm, with fructose solution') as the fixed factor. The analysis was performed using the R package survival (Therneau, 2021). We used the R packages survival (Therneau, 2021) and survminer (Kassambara and Kosinski, 2018) to plot survival curves. Further post-hoc analyses of differences between experimental treatments adjusted for multiple comparisons with the Benjamini-Hochberg method to control the false discovery rate (FDR) were implemented with the R package multcomp (Hothorn et al., 2008).

2.3.2. First instar mortality

To examine more closely the treatment effects on first instar mortality, generalised linear models (GLMs) with binomial error structure were used. GLMs were implemented using the stats R core package (R core team, 2021). Statistical significance of differences in mortality between the six experimental treatments were evaluated using FDR corrected post-hoc tests implemented with the R package multcomp (Hothorn et al., 2008).

2.3.3. Larval development time

Due to overdispersion, a GLM with quasiPoisson error structure was used to investigate the effects of the six experimental treatments on larval development time (before production of the cocoon). Statistical significance of differences in larval development time between treatments were evaluated using FDR corrected post-hoc tests implemented with the R package multcomp (Hothorn et al., 2008).

2.3.4. Within cocoon mortality

Generalised linear models (GLMs) with binomial error structure were used to investigate the effects of the six experimental treatments on the mortality that occurred between cocoon spinning and emergence. GLMs were implemented using the stats R core package (R core team, 2021). Statistical significance of differences in mortality between the six experimental treatments were evaluated using FDR corrected post-hoc tests implemented with the R package multcomp (Hothorn et al., 2008).

2.3.5. Cocoon weight

A linear model (LM) with a Gaussian error structure was used to investigate the effects of the six experimental treatments on the weight of individuals with the cocoon. Sex and its interaction with treatment were also used in the model as fixed factors. Statistical significance of differences in cocoon weight between the experimental treatments was evaluated using FDR corrected post-hoc tests implemented with the R package multcomp (Hothorn et al., 2008).

All model assumptions were checked visually and were found to conform to expectations (normally distributed residuals, homogeneity of variance, no outliers). All statistical analyses were conducted using R version 4.1.0 (R core team, 2021).

3. Results

3.1. First Experiment: Ingestion of sugar solution

All larvae (n = 10 hatchlings, n = 22 freshly moulted II instar, n = 18 freshly moulted III instar) ingested the sugar solution during the 24-hour treatment period. The hatchlings almost tripled their average weight after the intake of the sugar solution. The freshly moulted larvae increased their weight by 57% (II instar) and 26% (III instar) (see Table 1 for the absolute weights).

Table 1
Weight in μg (mcg) ± SE (standard error) of *Chrysoperla pallida* instars just before and after 24-hour access to D-fructose water solution.

	I instar newly hatched	II instar freshly moulted	III instar freshly moulted
Pre-feeding weight	123 ± 6*	473 ± 22	2.073 ± 79
Post-feeding weight	357 ± 5*	744 ± 35	3.278 ± 140

* Because we used repeated measurements to determine the weight of newly hatched I instars, we derived the SE(sw) from within-subject standard deviation, i.e. deviation of repeated measurements (Bland and Altman, 1996).

3.2. Second Experiment: Responses to diets with and without fructose.

3.2.1. Larval survival and development (from hatching to cocoon spinning)

We found a strong effect of experimental treatment on larval survival (coxph; $\chi^2 = 270.84$, df = 5, P < 0.001; Figs. 1 and 2). Survival rates in the treatments 'no provision' and with 'water only' (Treatments 1 and 2) were significantly lower than those in the four other treatments, all of which included nourishment, either fructose solution and/or mealworm (Treatments 3 to 6) (coxph; FDR corrected post hoc test, P < 0.001; Figs. 1 and 2). Between the first two treatments, the survival was lowest in Treatment 1, 'no provision' compared to Treatment 2, 'water only' (coxph; FDR corrected post hoc test, P = 0.0154; Figs. 1 and 2). Among the other treatments, survival in Treatment 4, 'mealworm only' was lower than with the fructose solution alone (Treatment 3) (coxph; FDR corrected post hoc test, P = 0.037; Figs. 1 and 2). Survival when larvae received mealworm with water or mealworm with fructose solution (Treatments 5 and 6) was intermediate between those when mealworm and fructose solution were each presented alone (Treatments 3 and 4).

Among all six treatments combined, deaths mainly occurred during the I instar (97.0%), while II and III instars constituted only 1.8% and 1.2% of the total mortality, respectively. Thus, because it was impossible to make a meaningful analysis with such small numbers of events in II and III instars, we analysed only the first instar mortality separately, where we again found a significant effect of experimental treatment (GLM; $\chi^2 = 274$, df = 5, P < 0.001; Fig. 3). The differences between treatments confirmed the results of the overall (all instars) survival analysis, except: i) the fructose solution alone (Treatment 3) yielded significantly lower mortality when compared to the mealworm with water (Treatment 5) (GLM; FDR corrected post hoc test, P = 0.039; Fig. 3); mortality in mealworm with fructose solution (Treatment 6) was significantly lower when compared to the mealworm only treatment (Treatment 4) (GLM; FDR corrected post hoc test, P = 0.039; Fig. 3).

We found a significant effect of experimental treatment on larval development time among the larvae that survived the various 24-hour treatment periods (GLM; $\chi^2 = 373.04$, df = 5, P < 0.001; Fig. 4). The longest larval development time occurred when both food and water were withheld for the 24 h after hatching and moulting (Treatment 1) followed in order, always significantly, by those with water only (Treatment 2), fructose solution alone (Treatment 3), mealworm only and mealworm with water (Treatments 4 and 5), and the fastest, those with both mealworm and the fructose solution (Treatment 6) (GLM; FDR corrected post hoc test, P < 0.05; Fig. 4).

3.2.2. Within cocoon development (from cocoon spinning to emergence)

We found no significant effect of experimental treatment on mortality within the cocoon (LM; F = 2.12, df = 5, P = 0.833; Fig. 2). We also found no significant effect of experimental treatment on the weight of the cocoon (LM; F = 1.59, df = 5, P = 0.161; Fig. 6). Although our results are consistent with earlier studies (e.g., Principi and Canard, 1984) that cocoon weight differs significantly between the two sexes (LM; F = 256.73, df = 1, P < 0.001), the interaction between sex and experimental treatment was not statistically significant (LM; F = 1.16, df = 5, P = 0.329).

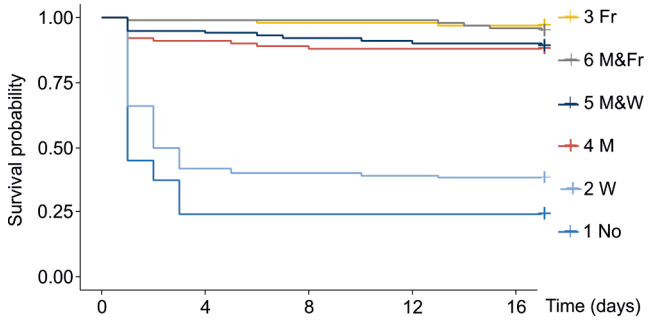

Fig. 1. Survival of larvae from hatching to cocoon spinning in each experimental treatment. 1 No = no provision, 2 W = water only, 3 Fr = fructose solution only, 4 M = mealworm only, 5 M&W = mealworm, with water, 6 M&Fr = mealworm, with fructose solution.

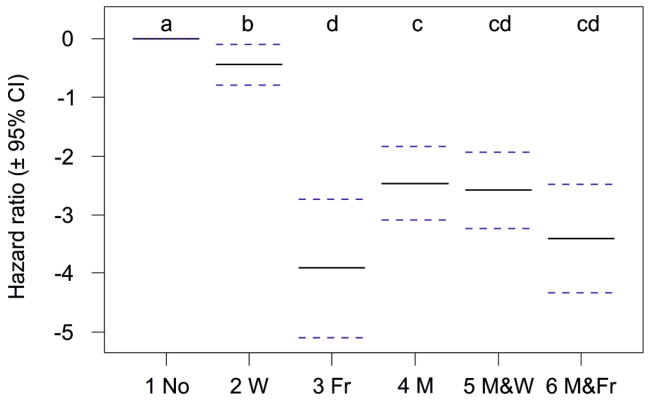

Fig. 2. Instantaneous risk of death (hazard ratio, ± 95% CI) in each experimental treatment compared with the model average of 0. Different letters correspond to significant differences between treatments at P < 0.05 (coxph and post-hoc pairwise contrasts adjusted for multiple comparisons with the Benjamini-Hochberg method to control the false discovery rate). 1 No = no provision, 2 W = water only, 3 Fr = fructose solution only, 4 M = mealworm only, 5 M&W = mealworm, with water, 6 M&Fr = mealworm, with fructose solution.

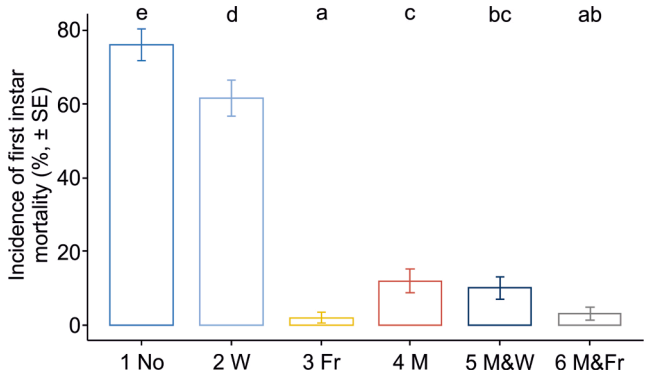

Fig. 3. Incidence of first instar mortality in each experimental treatment. Different letters correspond to significant differences between treatments at P < 0.05. 1 No = no provision, 2 W = water only, 3 Fr = fructose solution only, 4 M = mealworm only, 5 M&W = mealworm, with water, 6 M&Fr = mealworm, with fructose solution.

4. Discussion

4.1. Ingestion of a sugary drop

The weights of the three instars of *Chrysoperla pallida* fell within the known ranges of other species (Canard et al., 1996). All tested specimens of all instars accepted a droplet of fructose. The weight gains recorded just after larvae ingested the sugary solution were very high (Table 1). To the best of our knowledge, the only known weight increase after an intake of sugar solution is that reported by Hogervorst et al. (2008) for the II instar of *Chrysoperla carnea*. Their value of about a 40% increase is not far from ours; however, it is unknown whether their larvae had just moulted like ours had.

4.2. Effects of the sugary drop

Access to a fructose solution during three 24-hour periods

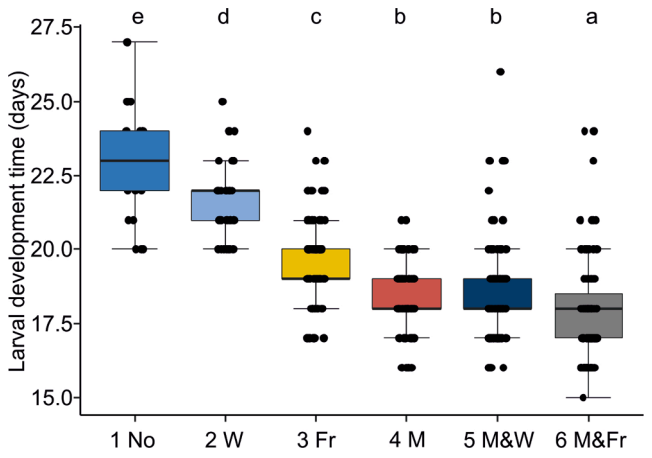

Fig. 4. Larval development time (from hatching to cocoon spinning) in each experimental treatment. Box plots show median and interquartile range. Different letters correspond to significant differences between treatments at $P < 0.05$. 1 No = no provision, 2 W = water only, 3 Fr = fructose solution only, 4 M = mealworm only, 5 M&W = mealworm, with water, 6 M&Fr = mealworm, with fructose solution.

(immediately after hatching and after each of the two larval moults) had significant positive effects on larval performance. That is, larvae that had access to the fructose solution alone (Treatment 3) were significantly less likely to die than those with no food or water (Treatment 1), water only (Treatment 2), or mealworm only (Treatment 4) (Figs. 1 and 2). The treatments also significantly influenced the development times (Fig. 4). Fastest development occurred when larvae had access to both mealworm and the fructose solution (Treatment 6). The regimen with fructose alone (Treatment 3) yielded developmental times that approached, but did not match the developmental times of the three treatments with mealworm (Treatments 4, 5, 6), i.e. those containing both protein and lipids.

The effects of ingesting a sugary solution relative to a factitious prey is the focal point of our results. With survival or risk of mortality as a measure, the intake of fructose solution during the first 24 h after hatching not only can substitute for a prey protein meal, but it also appears to provide additional benefits over prey alone. The fructose solution thus appears to fulfill a nutritional or other requirement. However, the question remains as to what kind of benefit a sugar solution provides. Most likely, the effects are complex with several components.

One possible, but weakly supported component is hydration. The provision of water (Treatment 2) somewhat mitigated the negative effects of provision deprivation (Treatment 1) on survival of first instars (and as a result, the overall rate of survival), and on development time. However, unlike the fructose solution, water did not appear to improve the value of a diet with mealworm as measured by developmental time. Indeed, the treatments of mealworm only (Treatment 4) and mealworm with water (Treatment 5) did not yield significantly different outcomes (Figs. 2 and 4). Thus, the benefits provided by the fructose solution in our experiment, as proposed by Limburg and Rosenheim (2001) for nectar, "extend beyond those provided by a simple water source".

A second, and more important, component could be the role of sugar as a source of energy. The treatment with fructose solution alone (Treatment 3) did not shorten developmental times below those from a diet of mealworm alone (Treatment 4), contrary to when it was combined with mealworm (Treatment 6). However, the treatment with fructose solution alone (Treatment 3) registered the lowest mortality in I instar. Evidently, the availability of fructose alone did not promote growth, but rather somatic maintenance. These results fit well with those obtained in previous research, which concluded that sugar alone does not lead to increased biomass, but sugar does promote searching activity and longevity (Limburg and Rosenheim, 2001; Hogervorst et al., 2008), and together with a protein source, it enhances the growth rate compared to that from a protein only diet (Patt et al., 2003).

It is noteworthy (and consistent with our argument above) that, despite the significant effects of the treatments on survival and developmental time, we did not detect any effects on the cocoon stage — neither on mortality within the cocoon nor on weight (Figs. 5 and 6).

4.3. An explanatory hypothesis.

The first hours after hatching constitute a critical period in the life history of a *Chrysoperla* larva. A small, thin, soft, and weak creature leaves its eggshell and immediately needs to become an aggressive predator capable of attacking and overwhelming a victim. If the neonate then encounters a prey, it will attack, in order to obtain a rich protein meal, an attempt that may or may not be successful. However, a less risky option is possible. The neonate larva could encounter a sugar meal such as a droplet of honeydew or nectar. Both sweet sources are common and abundant in nature, particularly if the adults choose appropriate sites for oviposition. The intake of a sugar droplet will provide the young larva with weight and energy, making it more capable of capturing and consuming prey. In other words, sugar extends the time, speed, and power of the hatchling's searching activity.

For predaceous larvae in general, hatchling size of predators may be constrained by the minimum size at which it is able to capture prey (Lamb and Smith, 1980; Stewart et al., 1991; Albuquerque et al., 1997). In addition, larger hatchlings may be more able to withstand periods of starvation at low prey densities (Lamb and Smith, 1980; Tauber et al., 1991). By relying on an external supply of sugar for weight and energy gain, *Chrysoperla* hatchlings may overcome the disadvantages of their small size, thus partially mitigating the trade-off between progeny size and number (Fox and Czesak, 2000).

5. Conclusion

The role of sugary, non-prey resources in the life history of chrysopid larvae is gradually becoming clearer (see the review of Albuquerque et al., 2012). The old paradigm of occasional omnivory that bridges periods of prey scarcity retains a certain validity, in the sense that it does happen; however, omnivory is anything but occasional. Sugary food, when available, is normally accepted and has been shown to enhance growth and quicken development, to make poor prey more suitable, and to improve searching activity (McEwen et al., 1993; Limburg and Rosenheim, 2001; Patt et al., 2003; Hogervorst et al., 2008). In other words, sugar works like a booster, an energetic fuel that strengthens important physiological functions. The role of sugary foods in somatic

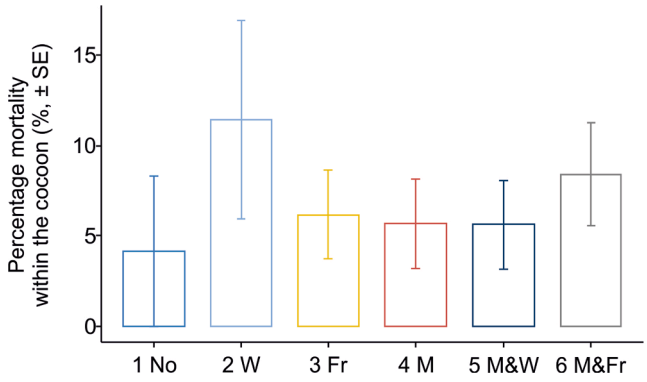

Fig. 5. Incidence of mortality within the cocoon (between spinning and adult emergence) in each experimental treatment. There are no significant differences between treatments at $P < 0.05$. 1 No = no provision, 2 W = water only, 3 Fr = fructose solution only, 4 M = mealworm only, 5 M&W = mealworm, with water, 6 M&Fr = mealworm, with fructose solution.

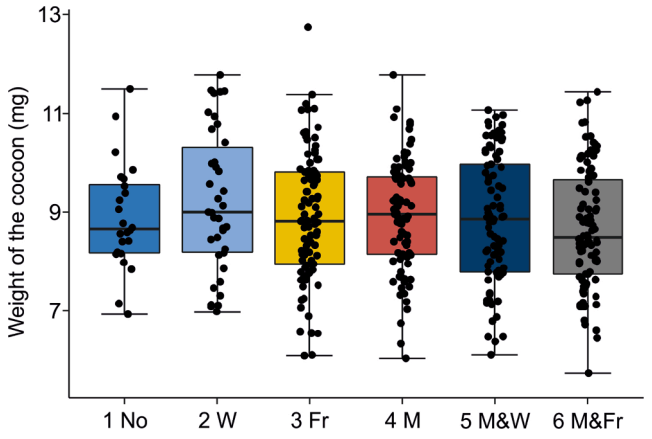

Fig. 6. Weight of the cocoon in each experimental treatment. Box plots show median and interquartile range. There are no significant differences between treatments at $P < 0.05$. 1 No = no provision, 2 W = water only, 3 Fr = fructose solution only, 4 M = mealworm only, 5 M&W = mealworm, with water, 6 M&Fr = mealworm, with fructose solution.

maintenance is important in many protein-feeding insects, such as hematophagous species (Yuval, 1992).

In this paper, for the first time the effects of sugar intake are linked to a precise period in the chrysopid's life history. The period after hatch is a very delicate moment, and a neonate larva that is able to find a drop of sweet food increases its life expectancy significantly. In fact, the first meal after hatchling has an influence on the long path of the individual's development.

These results have valuable practical application. First, providing a suitable sugary diet for hatchings could improve the efficiency and productivity of mass rearing and lab rearing methods. Second, in the context of conservation biological control, the awareness of the stage-specific benefit of a sugary diet could help refine some agro-ecosystem manipulation techniques such as floral resource augmentation or the application of artificial food sprays (Rayl et al., 2018).

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Roberto Antonio Pantaleoni: Conceptualization, Methodology, Resources, Writing – review & editing, Visualization, Supervision, Funding acquisition. **Michelina Pusceddu:** Data curation, Writing – original draft, Writing – review & editing. **Catherine A. Tauber:** Validation, Writing – review & editing. **Panagiotis Theodorou:** Formal analysis, Writing – review & editing. **Laura Loru:** Investigation, Resources, Writing – review & editing, Project administration, Funding acquisition.

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