**Food quality of the aphid Rhopalosiphum padi to the pirate bug Orius majusculus**

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<td>Toft, Soeren; Aarhus University, Department of Bioscience Jensen, Kim; Aarhus University, Department of Bioscience Sørensen, Jesper; Aarhus University, Department of Bioscience Sigsgaard, Lene; University of Copenhagen, Department of Plant and Environmental Science Holmstrup, Martin; Aarhus University, Department of Bioscience</td>
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Abstract

We studied the food quality of the aphid *Rhopalosiphum padi* to the pirate bug *Orius majusculus* compared with the factitious prey (*Ephestia* eggs) used by biocontrol companies. Several performance parameters were tested on individuals that had been reared and maintained on each of the two single-prey diets and on a mixed diet. All fitness parameters were lower in individuals fed aphids only, indicating poor food quality of this prey. Compared to the pure *Ephestia* egg diet, the mixed diet enhanced teneral mass, while adult survival and female starvation tolerance were negatively affected and all other traits were unaffected. Body protein proportions were constant across diets, whereas lipid proportion was low in the aphid treatment. Preference for aphids was lower following a monotypic aphid diet than when reared on *Ephestia* eggs or a mixed diet. The results imply little benefit of including *R. padi* as a priming prey in the developmental diet of mass-reared *O. majusculus* for controlling *R. padi*.

Key words: Aphididae, Anthocoridae, biological control, dietary mixing, life cycle, pest
Introduction

Generalist and specialist predators have different roles in the biological control of insect pests. Briefly, due to their narrow prey specificity and ability to track the populations of their focal prey species, specialists may be released in augmentative control programs to control an ongoing pest outbreak (Messenger, Wilson & Whitten, 1976). In contrast, generalist predators may subsist on alternative prey and be present in the fields before the pests arrive and thus may function in conservation control to prevent outbreaks (Riechert & Lockley, 1984; Symondson, Sunderland & Greenstone, 2002). Generalist predators are also mass produced by biocontrol companies for use in augmentative control against selected pests. Benefits include their ability to control more than one prey type and to survive on alternative prey or non-prey food, allowing preventative release strategies (see for example Messelink et al., 2015). The equivalent of prey specificity for generalist predators is the food quality of the prey, which may take any value from excellent to valueless or the prey may be toxic (Slansky & Scriber, 1985; Toft & Wise, 1999). Though the food value of a prey depends on its composition of nutrients and defensive chemicals, it cannot be evaluated solely from chemical analyses of prey composition. The same prey has different food value to different generalist predators depending on characteristics of the specific predator. Therefore, it is difficult to predict the outcomes of particular predator-prey interactions and each predator-prey combination must be studied directly. This is especially relevant for biocontrol systems that, by definition, try to manipulate such interactions for a specific goal, i.e. reduction of a particular pest population.

The food quality of prey to generalist predators is a central aspect of the predation process and thus an important, though under-appreciated, aspect of biocontrol (Thompson & Hagen, 1999). We can only expect a generalist predator to control a pest outbreak with the same
efficiency as a specialist if the pest is high-quality food. In spite of this, very little effort has been
made into evaluating and comparing the value of potential target pest species to specific
generalist predators, even those that are commercially available.

The pirate bug *Orius majusculus* Reuter (Hemiptera: Anthocoridae) is a generalist
predator, which is mass produced and sold for biocontrol purposes. It is usually reared with eggs
of *Ephestia kuehniella* Zeller (hereafter: *Ephestia* eggs) as factitious prey. Thrips are the main
target for control (Riudavets & Castañé, 1998; Blaeser, Sengonca & Zegula, 2004), but *O.*
*majusculus* or other *Orius* species have been tested for predation efficiency against a wide range
of arthropod pests (spider mites: Chysik, Klein & Ben-Dov, 1995; Venzon, Janssen Sabelis, 2002;
whiteflies: Montserrat, Albajes & Castañé, 2000; Arnó, Roig & Riudavets, 2008; leafhoppers and
lepidopteran larvae: Ardanuy, Albajes & Turlings, 2016; *Drosophila suzukii* eggs and larvae: Englert
& Herz, 2018). Aphids have frequently been considered potential targets of *Orius* spp. (Alvarado,
Báltà & Alomar, 1997; Brødsgaard & Enkegaard, 1997; Sengonca, Ahmadi & Blaeser, 2008;
In fact, Hodgson & Aveling (1988) considered *O. majusculus* as an important aphid predator in
Europe. A congeneric species, *O. insidiosus*, is considered an important natural enemy of the
soybean aphid *Aphis glycines* (Harwood et al., 2007). Some of these studies considered *Orius* spp.
as natural/conservation control agents, others as potential augmentative biocontrol agents.

Though recommended mostly for thrips control, this target prey may not be the highest quality
food. For *O. majusculus*, Husseini, Schumann & Sermann (1993) showed that the mould mite
*Tyrophagus putriscentiae* was of higher nutritional quality with respect to developmental rate and
nymphal survival than the target thrips species. Similarly, *Ephestia* eggs result in higher fecundity
and longevity than a thrips diet (Tommasini, van Lenteren & Burgio, 2004; Sobhy et al., 2010).
Generalist predators may provide significant natural control of arthropod pests of low food quality, whereas their effectiveness against such prey in augmentative release programs is doubtful. Thus, generalist predators may function as conservation control agents against aphids even if aphids consistently are low-quality food to such animals (Symondson et al., 2002; Toft, 2005). These generalist predators seem to maintain a low but consistent predation rate on the aphids due to repeated inductions of temporal aversions against low-quality prey (Bernays, 1993; Toft, 1997, 2005; Sunderland, 1999). This implies that they may impact aphids during the immigration phase to the fields when the aphids are still low in numbers, but not once the aphid populations have started to increase. Predatory Heteroptera were not included in the previous studies (Toft, 2005); other results indicate, however, that aphids may be considerably lower food quality to Orius spp. than thrips (Bonte et al., 2015). If confirmed for a wider range of aphid species, it would explain why they may be effective natural control agents of aphids while not recommended for augmentative control of these pests.

Because biocontrol agents used in practical biocontrol are often raised on a factitious prey, i.e. a prey or (artificial) food different from the target prey, the predator-prey relationship between the BCA and target pest may be influenced by the BCA’s experiences with the developmental prey/food, especially if developmental and target prey differ in food quality to the BCA. Food conditioning may be a means of enhancing the efficiency of BCAs, if their predation rate on a specific prey is increased when the BCA is raised on this prey or, if raised on a different prey/food, is primed for a short period on the prey prior to release (Ishii & Shimada, 2010). Examples of this effect are known from O. majusculus (Henaut, Alauzet, Ferran & Williams, 2000), Orius similis (Zhang, Zhi & Mo, 2013), ladybirds (Houck, 1986; Khan & Khan, 2002), predatory mites (Su et al., 2019), and parasitoids (Cascone et al., 2015). However, prey conditioning may also have
opposite effects, i.e. reduce the predation rate on the low-quality prey species (e.g. ladybeetles (Boivin, Roger, Coderre & Wajnberg, 2010) and chrysopid larvae (Nesbit, Wilby, Roberts & Menéndez, 2015)). Jensen et al. (2019) showed that experience with a risky collembolan prey in the predatory mite *Gaeolaelaps aculeifer* reduced its preference for that prey. Thus, results on the effects of prey conditioning of BCAs are mixed, and it is unclear why they are sometimes positive and sometimes negative with respect to enhancing the efficiency of the BCAs.

Here we compare the performance of *O. majusculus* under three developmental diet regimes: *Ephestia* eggs, the aphid *Rhopalosiphum padi* (L.), and a mixed diet of both. These aphids are natural prey of *O. majusculus* (Bokina, 2008), and we have observed abundant *R. padi* in maize fields where *O. majusculus* are numerous (Jensen, Toft, Sørensen & Holmstrup, submitted).

Several traits of importance for fitness and performance were included in the analysis. Our aims were 1) to evaluate the relative food quality of *R. padi* to *O. majusculus*; 2) to test if a diet more diverse than the monotypic one used in standard mass production of *O. majusculus* would create potentially more potent BCAs; and 3) to test if it is possible to create BCAs with a higher preference for the target prey by raising them on that prey.

**Materials and methods**

**Animals and general procedures**

We obtained *O. majusculus* from a commercial producer (EWH BioProduction, Tappernøje, Denmark) as large juveniles and young adults and founded a stock culture in the laboratory maintained on *Ephestia* eggs. Aphids (*R. padi*) were reared in the laboratory on wheat (*Triticum*
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105 *aestivum* seedlings. Laboratory rearing and experiments took place at room temperature (21 – 23
106 °C) and natural daylight conditions during March-May 2019. Individual *O. majusculus* were housed
107 in plastic vials (6 cm high, 2 cm diameter) closed by foam stoppers. Each vial contained a 4 cm
108 piece of bean (*Phaseolus vulgaris*) pod which provided structure and humidity, and served as egg
109 laying substrate in the reproduction experiment (Seagraves & Lundgren, 2010). Bean pods were
110 supplemented or exchanged at regular intervals to ensure a constant fresh substrate.

111 Because we wanted to test several performance parameters on the adult bugs, we
112 raised three separate batches of nymphs derived from the stock culture at different times during
113 the study period. Juvenile development time, juvenile survival, and teneral mass were measured
114 in all three batches. After reaching adulthood, the animals of the first batch were used to study
115 diet-dependent reproduction and adult survival. The animals of the second batch were first used
116 for a test of predation preference, then for measuring locomotor activity, and finally for measuring
117 body composition. Animals were left in the tubes with *ad libitum* access to their diet for a couple
118 of days between each of these measurements. Adults from the third batch were used for
119 measuring starvation tolerance. Following the moult to adults, individuals in all batches were
120 paired with another individual of the opposite sex from the same dietary treatment, and the pair
121 was kept under similar conditions as before including the same diet. If insufficient males were
122 available from a treatment, replacements were taken from the stock culture; these males were
123 not included in performance tests. Tests of prey preference, locomotory activity, body
124 composition and starvation tolerance were started after 7-10 days of adulthood, i.e. they were
125 measured on well-fed and reproductively active individuals.

126 *Juvenile development, juvenile survival and teneral mass*
Within 24 hours of hatching, first instar nymphs from the stock culture were transferred to bean pod pieces in individual vials and randomly assigned to the four dietary treatments: *Ephestia* eggs, *R. padi* aphids, mixed diet, or no prey. Since many anthocorids including *Orius* spp. suck plant juices as a dietary supplement to prey (Lattin, 1999; Pumariño & Alomar, 2012), the bean pods might also serve as a nutrient source; in fact, *O. insidiosus* may complete nymphal development on bean pods alone, though with low success compared with a prey diet (Richards & Schmidt, 1996). In order to test this possibility, the two first batches also included a “bean pod only” (control) treatment.

The vials were inspected approximately every second day throughout the duration of nymphal development and daily during the periods of the final moult, noting dead and moulted individuals. Fresh food was provisioned *ad libitum*, *Ephestia* eggs 1-2 times per week, aphids every 2-3 days. Aphids were served by providing wheat leaves with dense aphid colonies. Total development time was noted, and teneral mass was weighed within 24 h after the adult moult.

**Reproduction and adult survival**

Following establishment of adult pairs, survival was recorded every 2-3 days, and the bean pods were inspected for eggs under a binocular microscope. If eggs were present, they were counted and the bean pod was transferred to a separate tube for hatching of the eggs. A new piece of bean pod was provided for the pair. This was repeated until all individuals were dead or had stopped egg laying, thus providing information on lifetime reproduction of the females. The number of hatched eggs was recorded c. 8 days after collecting the pod by counting eggs with a lifted operculum. Total individual egg and offspring production were calculated by summing the number of eggs laid and the number of hatched eggs, respectively, from the total number of bean pods.
collected from each female. Hatching success per female was calculated as the proportion of hatched eggs from the total number of eggs laid.

When the pairs were established, there was a surplus of males in the *Ephestia egg* treatment (*n* = 12). These males remained in their individual tubes and were fed *Ephestia eggs ad libitum*. Their survival was compared with that of the reproducing males on the *Ephestia egg* treatment, providing an estimate of male survival costs of reproduction.

**Body composition**

Our analysis procedure allows us to distinguish three fractions of the body: lipid, exoskeleton, and protein. The latter fraction excludes protein bound in the exoskeleton, but includes minor body constituents such as carbohydrates, nucleic acids and others. The procedure was as follows. The animals were freeze-killed, dried in a vacuum oven (VacuTherm VT6060M, Thermo Scientific, Langenselbold, Germany) at 60 °C for two days, and total body dry mass was weighed to the nearest µg. Lipid was then extracted by soaking carcasses in three successive 48 hour baths of 0.5 mL petroleum ether. After drying, the carcasses were weighed again, and body lipid content was calculated as the mass difference before and after extraction. The lean carcasses were then soaked in three 48 hour baths of 0.35M NaOH (Marden, 1987; Rho & Lee, 2014; Poissonnier, Arganda, Simpson, Dussutour & Buhl, 2018), which removes the remaining soft tissue, primarily consisting of proteins, and leaves the exoskeleton. This was cleaned in a 48 hour bath of demineralized water, then dried and weighed, and protein mass was estimated as the difference between lipid-free carcass mass and exoskeleton mass.

**Starvation tolerance**
After the seven days of adulthood in pairs, the animals were separated and kept individually in a tube with pieces of bean pod for maintaining humidity and egg laying, but no food was provisioned. Individuals were checked for survival daily, and fresh bean pods were added if needed. Animals that died before the start of the starvation treatment were not included in the analyses.

**Locomotor activity**

The apparatus for measuring locomotor activity consisted of 3 stacked plexiglass plates (2 mm thick) of which the middle plate had 36 drilled holes (diameter 2 cm) forming chambers for the animals. Two such units were operated at the same time. One adult *O. majusculus* was released into each of the 72 chambers. After approximately two hours, the animals’ activity was video recorded by means of a Huawei mobile phone for approximately 1 hour. The recordings were analyzed by Ethovision tracking software (EthoVision XT v.10, Noldus, Wageningen, The Netherlands). We used the total distance covered by each animal as the measure for activity. One animal was damaged and therefore excluded; thus $N = 71$ (41 females, 30 males) distributed among the three diets (*Ephestia* eggs 25, aphids 25, mixed diet 21).

**Prey preference**

The experiments were run in glass tubes (1 cm diameter, 5.5 cm deep) to which were added 2 *Ephestia* eggs and two small *R. padi* aphids (instar I-II, only slightly larger than *Ephestia* eggs). We noted the time at which each bug was released into the tube, the time of first prey capture, and whether an egg or an aphid was chosen. The animal was then disturbed to prevent any prey consumption and returned to its rearing tube. Sample sizes were: *Ephestia* eggs-reared bugs 27; aphid-reared bugs 18; mixed diet-reared bugs 19.
Statistical analyses

Data on three parameters (duration of juvenile development, juvenile survival and teneral mass) were obtained from all three batches (“blocks”) of animals raised at different times. In preliminary analyses, we therefore included block and treatment*block effects. The block effect was maintained in the final analysis if significant, otherwise excluded.

For analyzing male survival, we used both the Wilcoxon test and the Log-Rank test (Pyke & Thompson, 1986). By combining the two tests we can distinguish early from late mortality, since the former test is most sensitive to the early part of the survivorship curve, while the latter is most sensitive to its late part (Pyke & Thompson, 1986). Other survival data (juveniles, adults, starvation tolerance) were analyzed using the Wald test in a proportional hazard analysis (Cox, 1975; Bangdiwala, 1989). Not all individuals were seen during every inspection; if they were never resighted or found dead, we used the day after the last observation of the animal alive as the day of death. This was also the case for first instar nymphs that were never seen after experiment start, i.e. survival = 1 day. Individuals that reached adulthood were censored and the date of the moult to adult was included. Pairwise comparison of treatments were made using risk ratios.

Data on several of the other life-history parameters and body composition did not fulfil the assumptions of parametric tests and could not be transformed appropriately. We therefore used the non-parametric proportional hazard analysis also for them.

Juvenile survival was analyzed with diet treatment as the only factor; for developmental duration, sex and block were added as factors; for teneral mass, developmental duration was included as covariate. For fecundity, we included teneral mass as covariate. In some analyses, sex and mass were significantly correlated co-factors; in such cases, we maintained sex
as a factor but excluded mass. All statistical analyses were made with JMP v. 13.0 (SAS Institute Inc., Cary, NC, USA).

Results

Survival during juvenile development

Juvenile survival (Fig. 1) depended significantly on diet treatment (Wald test: $\chi^2_3 = 113.5, P < 0.0001; n = 712$). Animals of the control treatment (“bean pod only”) survived very poorly; most of them died in the first instar while only a few moulted a single time. Compared with animals of the other treatments, they had a pronounced immediate mortality, indicating that they were starved. Pumariño & Alomar (2012) also found that adult female *O. majusculus* survived very badly when offered only bean pods and that bean pods did not contribute to fecundity. Individuals on the *Ephestia* egg diet and the mixed diet survived equally well and significantly better than individuals on the pure aphid diet (post-hoc tests: *Ephestia* eggs = Mixed diet < Aphid diet; Fig. 1).

Juvenile developmental duration

Duration of nymphal development for individuals that reached adulthood depended on both diet (Wald test: $\chi^2_3 = 43.6, P = < 0.0001; n = 341$) and sex ($\chi^2_1 = 5.8, P = 0.0165$), and differed between blocks ($\chi^2_2 = 70.5, P < 0.0001$). The block effect was a gradual shortening of developmental duration in all combinations of treatment and sex (data not shown), probably due to increasing daylength during the experimental period (Van den Meiracker, 1994; Bahşi & Tunç, 2012). The combined results (Fig. 2A) show that individuals on the *Ephestia* egg and mixed diet had the shortest developmental durations, while individuals fed only aphids took significantly longer to
develop (post-hoc tests: *Ephestia* eggs = Mixed diet < Aphid diet). Males matured slightly faster than females in all treatments.

**Teneral mass**

Teneral mass (Fig. 2B) depended on developmental diet (Wald test: $\chi^2_2 = 84.6, P < 0.0001; n = 321$) and sex ($\chi^2_1 = 167.0, P < 0.0001$), with a significant diet*sex interaction ($\chi^2_2 = 6.8, P = 0.034$) and a negative correlation with developmental time ($\chi^2_1 = 15.9, P < 0.0001$); further, there was a barely significant block effect ($\chi^2_2 = 6.2, P = 0.044$). Teneral mass differed significantly between all diets with the highest mass following development on the mixed diet and the lowest following development on aphids only. Adult females were heavier than males within all diets, especially in the mixed diet treatment. The negative correlation shows that teneral mass decreased with developmental duration (data not shown), i.e. slowly developing individuals became smaller adults than fast developing ones. The block effect was one of increasing mass as would be expected from its negative effect on developmental duration and the negative relationship between teneral mass and developmental duration.

**Adult survival during reproduction**

Both diet (Wald test: $\chi^2_2 = 38.6, P < 0.0001; n = 128$) and sex ($\chi^2_1 = 7.9, P = 0.0049$) affected adult survival with no significant interaction ($\chi^2_2 = 1.3, P = 0.53$). Survival differed significantly between all diets with highest survival on *Ephestia* eggs and lowest on aphids, a pattern repeated in both sexes (Fig. 3). Females survived longer than males. These results indicate a negative effect of the mixed diet compared to *Ephestia* eggs only.
Comparison of mated and unmated males (Fig. 4) shows an early mortality which was independent of mating status (Wilcoxon test $\chi^2_1 = 1.65, P = 0.20$); however, longevity was significantly lower in mated than unmated males on a longer term (Log-rank test $\chi^2_1 = 5.08, P = 0.024$), separating at around 30 days of adulthood (Fig. 4).

Fecundity and hatching success

Fecundity was much lower in the aphid treatment compared with the *Ephestia* egg and mixed diet treatments (Fig. 5A). Statistical test with only diet as factor revealed a highly significant diet effect (Wald $\chi^2_2 = 15.2, P = 0.0005$; $n = 46$) with *Ephestia* eggs = Mixed diet > Aphid diet. Including teneral mass as covariate, however, gave a highly significant size effect ($\chi^2_1 = 15.5, P < 0.0001$) but removed the diet effect ($\chi^2_2 = 1.0, P = 0.60$). Thus, the diet effect was at least in part explained by the lower mass of animals maintained on aphids. Hatching success showed a similar pattern being high on the *Ephestia* egg diet and mixed diet and lower for females maintained on aphids only (Fig. 5B; Wald $\chi^2_2 = 10.8, P = 0.0046$; $n = 32$), but was independent of teneral mass ($\chi^2_2 = 1.1, P = 0.29$).

Body composition

We illustrate the proportional body composition in relation to treatment and sex by means of the right-angled mixture triangle (RMT; Raubenheimer, 2011). RMT allows us to plot 3-dimensional data in two dimensions (Fig. 6): we show the relative protein content on the abscissa and the relative lipid content on the ordinate. Since the proportional contents of the three fractions sum to 1, there is an implicit axis from the diagonal line between (0, 1) and (1, 0), increasing towards a maximum of 1 at the origin (0, 0) of the coordinate system. The proportional exoskeleton content can be read as the distance from this diagonal to the (protein, lipid)-point in the plot.
The proportional protein content in the body (Fig. 6) was similar in all diet treatments (Wald test: $\chi^2_1 = 3.95, P = 0.139; n = 108$), in both sexes ($\chi^2_1 = 0.39, P = 0.534$) and independent of dry body mass ($\chi^2_1 = 1.81, P = 0.178$) and all interactions between these factors. In contrast, the proportion of body lipid was strongly dependent on treatment ($\chi^2_2 = 45.5, P < 0.0001$), sex ($\chi^2_2 = 29.6, P < 0.0001$) and treatment*sex interaction (Wald test: $\chi^2_2 = 9.5, P = 0.0086$). The relative lipid content of bugs from the aphid treatments were lower than predicted by their mass (ANOVA on residuals from a regression between proportional lipid content and dry body mass: treatment $F_2 = 10.4, P < 0.0001, n = 102$, sex $F_1 = 0.03, P = 0.86$, treatment*sex $F_2 = 1.83, P = 0.17$; least squares means: *Ephestia* eggs 0.012, Mixed diet 0.017, Aphid diet -0.067). The proportion of exoskeleton was negatively related to dry body mass (Wald test: $\chi^2_2 = 4.59, P = 0.032$), and negatively correlated with proportional lipid content ($t_{110} = -3.64, P = 0.0004$). Overall, the relative lipid content varied much while protein was constant and exoskeleton contents varied as a consequence of variation in lipid content.

**Starvation tolerance**

Survival under starvation (Fig. 7) depended on diet treatment (Wald test: $\chi^2_1 = 13.8, P = 0.0010; n = 100$) and sex ($\chi^2_1 = 9.2, P = 0.0024$) but was independent of the treatment*sex interaction ($\chi^2_1 = 1.7, P = 0.42$) and teneral mass ($\chi^2_1 = 0.5, P = 0.47$). Survival was lower after maintenance on aphids only, but did not differ significantly after maintenance on the two diets including *Ephestia* eggs within both females (Fig. 7A) and males (Fig. 7B). Females survived starvation longer than males (Fig. 7).

**Locomotor activity**
Activity (Fig. 8) was considerably higher in males than in females (Proportional Hazards test, sex: Wald $\chi^2_1 = 18.6, P < 0.0001; n = 71$), but was not differently affected by diet (diet Wald $\chi^2_2 = 0.17, P = 0.92$; diet*sex Wald $\chi^2_2 = 1.1, P = 0.58$).

**Prey preference**

Females raised on *R. padi* aphids selected equal numbers of *Ephestia* eggs (E) and aphids (A) (9 E, 9 A), while females raised on *Ephestia* eggs or on the mixed diet selected aphids more often than *Ephestia* eggs (*Ephestia* egg treatment: 4 E, 15 A; mixed diet treatment: 7 E, 20 A) (Fig. 9).

Combined, females reared on *Ephestia* eggs and mixed diet showed a significant preference for aphids over *Ephestia* eggs (Yates test: $\chi^2_1 = 5.65, P = 0.018$), while females raised on aphids showed no preference and thus a higher immediate preference for *Ephestia* eggs than females from the other two diets. Preference in individuals from the combined *Ephestia* egg + mixed diet treatments was significantly different from that of individuals reared on aphids only (Pearson $\chi^2_1 = 4.32, P = 0.038$).

The time until prey capture was not affected by prey type (Wald test: $\chi^2_1 = 2.80, P = 0.095; n = 65$), but depended on rearing diet (aphid vs. *Ephestia* eggs + mixed diet: $\chi^2_1 = 4.34, P = 0.037$). Females reared only on aphids took longer to select a prey than females of the combined *Ephestia* egg + mixed diets (median/25% quartile: 4.25/1.50 vs. 2.00/1.00 minutes).

**Discussion**

**Overall findings**
The results revealed that *R. padi* aphids are low quality food for *O. majusculus*. The predator showed reduced performance in all fitness parameters tested when aphids were a monotypic diet. The aphids were not detrimental food, however. Nymphal development was completed by c. 40% of the nymphs raised on aphids alone (compared with 65% of the nymphs fed diets including *Ephestia* eggs). Reproduction, on the other hand, was considerably reduced when reared only on aphids due both to low fecundity and low hatching success. The aphids may be considered as supplementary prey, i.e. prey that promotes some fitness benefit as a supplement but is suboptimal on its own. Adding these aphids to the staple *Ephestia* egg diet thus had positive, negative or no consequences depending on the specific performance parameter. The diverging effects of a mixed diet on different parameters in the same species is a warning not to make strong conclusions in studies where only single or few parameters are studied. The food value of a prey species in mixed diets also depends on the quality/nutritional composition of the other species in the mix. For instance, in the sheet-web spider *Dicymbium brevisetosum*, *R. padi* was a positive supplement to the diet by increasing offspring survival if the staple prey had low nutritional quality, but not if it was nutritionally enriched (Bilde & Toft, 2000). Whether *O. majusculus* benefits overall from including the aphid in the diet, in mass rearing or in the field, cannot be finally concluded from the present results.

**Diet-dependent performance**

In spite of negative effects of the aphid as monotypic diet, teneral mass was positively affected by the addition of aphids in the mixed diet, which resulted in the heaviest bugs among both sexes. The higher teneral mass of individuals from the mixed diet suggests that the aphids contain (micro)nutrients that are missing or present in low amounts in *Ephestia* eggs but which are
beneficial to the predator, and it is not an indication that the aphids are generally beneficial for
growth. The ladybeetle *Harmonia axyridis* also showed higher developmental mortality and lower
teneral mass when reared on pea aphids compared with *Ephestia* eggs (Specty et al., 2003). The
high food quality of *Ephestia* eggs was related to a higher content of protein and fatty acids and a
lower content of glycogen. The ladybirds developed a difference in content of these
macronutrients corresponding to the differences between prey species (Specty et al., 2003). Most
likely, *R. padi* differs from *Ephestia* eggs in a similar way as pea aphids.

In addition to dietary effects on teneral mass, we found a general reduction in
teneral mass for slowly maturing individuals within dietary treatments. This effect was not only
found for individuals raised on aphids, but was repeated on each diet in both sexes, and reflects
individual differences in vigour (Klingenberg & Spence, 1997). Therefore, the pattern is of interest
also in relation to production of BCAs. Probably, the large fast maturing individuals are also the
most voracious and the most efficient BCAs, and they are the ones producing the most offspring.
Though genetic correlations between development time and body size in other heteropterans
seem weak (Klingenberg & Spence, 1997), consistent use of fast maturing individuals for
maintaining the production cultures may pay off. Such selection may occur simply as a
consequence of mass rearing practices.

The pure aphid diet had two major structural effects with consequences for fitness,
i.e. a reduced body mass and a low lipid content. The two effects seem to be (partly) independent
as the proportional lipid content of aphid-reared bugs was reduced relative to their body mass. A
decreased mass of females from the aphid treatment should result in lowered reproductive output
(Honěk, 1993). Accordingly, we found that fecundity depended on female mass and that females
of the aphid treatment had lower fecundity than females from other diet treatments. Oogenesis in insects is particularly dependent on protein resources (Wheeler, 1996). As the relative protein content was not lower in females reared on aphids, we assume that their low fecundity was caused by their small size combined with very low lipid stores that did not provide sufficient energy for egg production. Also the lower starvation tolerance of bugs reared on aphids only is probably a consequence of low lipid content. Jensen, Mayntz, Wang, Simpson & Overgaard (2010) found that wolf spiders fed lipid-rich/protein-poor flies had a higher lipid content and survived starvation longer than spiders fed lipid-poor/protein-rich flies. Similarly, Jensen et al. (2018) related enhanced starvation tolerance in cold-acclimated bugs (O. majusculus) and predatory mites (Gaeolaelaps aculeifer) to larger lipid stores induced by cold treatment. These studies support our finding that larger lipid stores from high-quality prey enhances starvation tolerance.

The low reproductive life of bugs from the aphid diet may have a similar explanation. Whether this can be extended to the reduced adult survival of bugs from the mixed diet treatment compared with those reared on Ephestia eggs is doubtful, since both groups had similar lipid stores and reproductive output. Alternatively, it may be due to high metabolic costs associated with feeding on the aphid. Thus, Toft & Nielsen (1997) found increased metabolic rate of wolf spiders fed R. padi compared with spiders fed fruit flies.

As the only performance parameter measured, locomotory activity was not reduced in the aphid-only treatment. Presumably, locomotor activity does not reflect the nutritional status of the animals, as they may use food searching activity in an attempt to alleviate a low body condition.
Maximal lifespan of *O. majusculus* males was considerably longer if not allowed to mate, indicating that mating is costly to the males. Leon-Beck & Coll (2009) found a similar effect on females of *O. laevigatus* but did not look for male costs. The mating system of *O. majusculus* seems not to have been investigated. In other *Orius* species, females may be monandrous or polyandrous, while the males of all species are polygynous (Leon-Beck & Coll, 2009; Arakawa et al., 2019). Therefore, we cannot tell whether male longevity costs resulted from single or multiple mating. Significant survival reductions in males due to a single mating have been found in several arthropod species, e.g. the dung beetle *Onthophagus binodis* (Kotiaho & Simmons, 2004) and the sepsid fly *Saltella sphondylli* (Martin & Hosken, 2003). In the coccinellid *Adalia bipunctata*, Perry & Tse (2013) found a 53% reduction in post-mating lifespan from a single mating.

**Prey preference**

We found an overall higher preference for aphids than for *Ephestia* eggs under choice, confirming that prey preferences do not necessarily reflect the food quality of the prey (e.g. Nedvěd & Salvucci, 2008). The shorter prey acceptance latency of the *Ephestia* egg+mixed diet group compared with the aphid diet group, rules out the possibility that attack was determined by hunger, thus providing evidence that prey preferences had been modified by the developmental diets. Several factors may have contributed to this outcome. Firstly, as prey is detected mainly by touch in anthocorids (Hodgson & Aveling, 1988), attack depending on encounter rate would, all other things equal, lead to no preference in the present experiment. Aphids are live prey, however, which may be more easily detected and identified (“encountered”) by a predator. Secondly, the groups of bugs that preferred aphids were well nourished, i.e. in good condition and presumably in nutritional balance, and are therefore expected to accept non-deterrent potential
prey that might provision deficient micronutrients. In contrast, aphid reared bugs were in bad
condition which would only be worsened by eating more aphids. Both groups showed an elevated
preference for the missing or less important ingredient of their developmental diet. In this
interpretation, our results suggest a nutritional explanation for different prey preferences
between aphid-reared and *Ephestia* egg+mixed diet-reared bugs. Nutritional self-selection
(Waldbauer & Friedman, 1991) allows such condition-dependent prey selection, which may lead to
intermittent preference for overall low-quality prey. In another study on *O. majusculus*, Henaut et
al. (2000) found that developmental diet (*Ephestia* eggs vs. pea aphids) enhanced adult preference
for the same prey, opposite to our findings. The reason for this difference of the two studies may
be found in the size of the aphid prey. Henaut et al. (2000) used 4\textsuperscript{th} instar aphids of approximately
the same size as the bug and observed successful anti-predator behavior (kicking); we used 1\textsuperscript{st} and
2\textsuperscript{nd} instar *R. padi*, only slightly larger than an *Ephestia* egg. Obviously, the small aphids did not
present a danger to the bugs and could not repel them as indicated by the short attack times (2-4
minutes); in fact, no case of successful anti-predatory behavior was observed. Our prey selection
results thus contradicted the expected enhancement of preference through prey conditioning.

**Implications for biological control**

Overall, results on using prey conditioning or priming to enhance biocontrol are inconsistent for
reasons that are still unclear. We suggest that the food quality of the pest may determine whether
developmental conditioning (or pre-release priming) will be beneficial for control efficiency: it is
more likely to be positive if the pest is high-quality food to the BCA, and more likely to be negative
if the pest is a low-quality, risky or toxic prey. If this or another general relationship can be
established, a more successful use of prey conditioning/priming would be possible. If the pest is
nutritionally imbalanced for the BCA, conditioning with a nutritionally complementary prey may be advantageous. To our knowledge, this has never been attempted in practical biocontrol. If the target pest is low-quality food to the BCA due to chemical defences, it may be better to leave the BCA naïve so the predation attempts during which the predator gains information about the quality of the prey (and develop an aversion against it; Bernays, 1993) will contribute to control.

Conclusion

The general conclusion that aphids are low-quality food for generalist predators (Toft, 2005), implying that the predators cannot maximize fitness-related life-history parameters by feeding on aphids alone, is here extended to the heteroperan O. majusculus. As found for other generalist predators, this does not mean that the aphid cannot contribute positively to the nutrition of O. majusculus, and it also does not mean that this predator is necessarily ineffective as a BCA against aphids, at least as a conservation control agent. Compared with the spiders and predatory beetles of agricultural systems, which mostly inhabit the ground surface habitat and therefore prey on aphids moving on the ground, Orius spp. and other predatory heteropterans live on the plants (Lattin, 1999) and thus may harvest directly from the growing aphid colonies. This may give them a key role in the early season assemblage of aphidophagous predators on plants.

Conflicts of interest The authors declare that they have no conflicts of interest.

Authorship statement

ST, KJ, JGS, LS and MH together conceived the study; ST conducted the experiments; ST and JGS analyzed the data; ST made the statistical analyses; all authors contributed to the manuscript and approved the final version.

Data Availability Statement
Data will be deposited in Dryad ... if the manuscript is accepted.

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Figure legends

Fig. 1. Survival during nymphal development in *Orius majusculus* raised on *Ephestia* eggs, aphids (*Rhopalosiphum padi*), a mixed diet of the two, or a no-food (starved) control. Curves with the same letter are not significantly different. Censored individuals reached adulthood.

Fig. 2. (A) Duration of nymphal development and (B) teneral mass of individual female *Orius majusculus* provisioned through their entire development and adulthood with *Ephestia* eggs (Eph), the aphid *Rhopalosiphum padi* (Aph), or a mixed diet of the two (Mix). Same letter above boxes indicate no significant difference between diet treatments within plots. The block effect is neglected in (A). Box plots show the median (horizontal line within boxes), 25 and 75 % quartiles (boxes), 10 and 90 % quantiles (bars), and outliers (points).
Fig. 3. Adult longevity (time since the maturity moult) of (A) female and (B) male *Orius majusculus* reared and maintained during adulthood on *Ephestia* eggs, aphids (*Rhopalosiphum padi*), or a mixed diet of the two. Within each sex, all curves are significantly different (indicated by different letters).

Fig. 4. Adult longevity (time since the maturity moult) of unmated and mated male *Orius majusculus*; the latter included males fed *Ephestia* eggs in the fecundity experiment. Mated males were paired with a female immediately after becoming adults.

Fig. 5. (A) total life-time egg production and (B) offspring hatching success of individual female *Orius majusculus* provisioned through their entire development and adulthood with *Ephestia* eggs (Eph), the aphid *Rhopalosiphum padi* (Aph), or a mixed diet of the two (Mix). Same letter above boxes indicate no significant difference between diet treatments within plots. Box plots show the median (horizontal line within boxes), 25 and 75 % quartiles (boxes), 10 and 90 % quantiles (bars), and outliers (points).

Fig. 6. Right-angled mixture triangle plot showing the proportional body content of adult *Orius majusculus* females (f) and males (m) raised on and subsequently fed one of three diets: *Ephestia* eggs (E), the aphid *Rhopalosiphum padi* (A), or a mixed diet of the two (M). Since the three body fractions sum to 1, the body proportion constituting the exoskeleton can be read as the distance from the points to the (0,1)-(1,0)-diagonal line, i.e. it increases from the diagonal line to reach the maximum value 1 at the origin (0,0).

Fig. 7. Starvation tolerance (survivorship) curves for starved adult (A) female and (B) male *Orius majusculus* reared on one of three diets: *Ephestia* eggs, aphids (*Rhopalosiphum padi*), or a mixed diet of the two. Curves with the same letter are not significantly different.
Fig. 8. Activity (meters walked over one hour) of adult (A) female and (B) male *Orius majusculus* reared on one of three diets: *Ephestia* eggs (Eph), the aphid *Rhopalosiphum padi* (Aph), or a mixed diet of the two (Mix). Box plots show the median (horizontal line within boxes), 25 and 75 % quartiles (boxes), 10 and 90 % quantiles (bars), and outliers (points).

Fig. 9. Prey selection by female *Orius majusculus* reared on one of three diets: *Ephestia* eggs (Eph), the aphid *Rhopalosiphum padi* (Aph), or a mixed diet of the two (Mix).
A.

Females

- Eph
- Mix
- Aph

Males

- Eph
- Mix
- Aph

Duration of nymphal development (days)

B.

Females

- Eph
- Mix
- Aph

Males

- Eph
- Mix
- Aph

Teneral mass (mg)
Survival of Ephestia eggs on different diets.

A. Females
B. Males

- Ephesia eggs
- Mixed diet
- Aphids

Survival (%) over 50 days.
Fecundity (eggs/female)

A.

<table>
<thead>
<tr>
<th>Eph</th>
<th>Mix</th>
<th>Aph</th>
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<tbody>
<tr>
<td>1.0</td>
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<td>0.4</td>
<td>0.2</td>
<td>0.0</td>
</tr>
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B.

<table>
<thead>
<tr>
<th>Eph</th>
<th>Mix</th>
<th>Aph</th>
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<tbody>
<tr>
<td>a</td>
<td>a</td>
<td>b</td>
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</table>

Hatching success

A. Eph, Mix, and Aph are significantly different from each other.

B. Eph, Mix, and Aph are significantly different from each other.
Proportion protein

Proportion lipid

f: females
m: males

Proportion exoskeleton

E-f
M-f
A-f
E-m
M-m
A-m

E: Ephhestia eggs
M: Mixed diet
A: Aphids

0.0 0.2 0.4 0.6 0.8 1.0
Proportion protein

0.0 0.2 0.4 0.6 0.8 1.0
Proportion lipid
Ephestia eggs
Mixed diet
Aphids

A. Females
B. Males

Survival
Days

0.0
0.2
0.4
0.6
0.8
1.0

0.0
0.2
0.4
0.6
0.8
1.0

Survival
Days

0.0
0.2
0.4
0.6
0.8
1.0

0.0
0.2
0.4
0.6
0.8
1.0

0.0
0.2
0.4
0.6
0.8
1.0

0.0
0.2
0.4
0.6
0.8
1.0
Prey selected:

- Ephesia eggs
- Aphids

% selected

0 20 40 60 80 100

Developmental diet

Eph Mix Aph

% selected

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