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## Cryptic species *Chrysoperla lucasina* and *C. carnea* differ in predation of early *Nezara viridula* instars

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Invasive pests increasingly threaten the stability of integrated pest management programs in greenhouse cropping systems, where biological control strategies are typically tailored to established pest complexes. *Nezara viridula* (Hemiptera: Pentatomidae) has emerged as a major pest in European greenhouses, where current biocontrol programs are poorly equipped to suppress it. Eight commercially available generalist arthropod predators commonly used in greenhouse solanaceous crops in Europe were assessed for their ability to suppress *N. viridula*. Their impact on its eggs and early nymphal instars was assessed through standardized laboratory assays, as well as greenhouse cage trials using fruit-bearing sweet pepper plants. Predation was observed mainly among larvae of *Chrysoperla* species (Neuroptera: Chrysopidae), with *C. lucasina* consistently outperforming *C. carnea*. Notably, *C. lucasina* was the only predator to attack *N. viridula* eggs, albeit at low levels. Against nymphal instars, third-instar *C. lucasina* larvae exhibited the highest predation, while second-instar *C. lucasina* often outperformed second-instar *C. carnea* and sometimes even matched the efficacy of third-instar *C. carnea*. All other predators showed little or no predation. In greenhouse cage trials, third-instar *C. lucasina* significantly reduced the survival of both first- and second-instar *N. viridula* nymphs under the structurally complex on-plant conditions. Across all assays, first-instar *N. viridula* nymphs were generally more vulnerable to predation than second-instar nymphs. These results identify *C. lucasina* as a promising candidate for augmentative biological control of *N. viridula*, warranting further evaluation under longer exposure periods, mixed-prey environments, and in combination with other biocontrol agents, such as egg parasitoids. Importantly, this study highlights how cryptic species within commercially used predators can differ markedly in efficacy, an underrecognized aspect of functional diversity in biological control.

**Keywords** Biological control, Integrated pest management, Stink bugs, Hemiptera, Lacewings, Chrysopidae

Biological control has become a cornerstone of integrated pest management (IPM) in greenhouse horticulture, where high-intensity, year-round production demands reliable and sustainable pest management. The deployment of natural enemies such as predators, parasitoids, and entomopathogens has enabled effective control of established pest complexes while reducing dependence on chemical inputs<sup>1–5</sup>. However, the globalization of trade has facilitated the introduction of invasive pests that escape these biocontrol frameworks and expose critical vulnerabilities in existing IPM programs<sup>6</sup>. Among these, *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Pentatomidae) has emerged as a persistent and damaging pest in European greenhouse crops, particularly sweet pepper (*Capsicum annuum* L.)<sup>7</sup>. Current biological control systems are poorly adapted to suppress *N. viridula*, and infestations often trigger chemical interventions that disrupt resident natural enemies and jeopardize long-term control stability<sup>8,9</sup>.

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In European protected cultivation, diverse assemblages of natural enemies have been commercialized and successfully implemented against key pests such as whiteflies, thrips, aphids, and lepidopterans<sup>7,10</sup>. However, the introduction of *N. viridula* exposes clear limitations in these established programs, as many existing natural enemies fail to control this invasive pest. Among the most widely deployed biological control agents, the egg parasitoid *Trissolcus basalis* (Wollaston, 1858) (Hymenoptera: Scelionidae) targets *N. viridula* eggs with high specificity<sup>9</sup>, yet field studies have reported incomplete parasitism of egg batches<sup>11</sup>, and population models suggest that parasitoids alone are insufficient to suppress stink bug populations<sup>12</sup>. These findings are consistent with observations in greenhouse settings, where substantial numbers of eggs often escape parasitism. As a result, many nymphs still emerge, thereby sustaining or increasing infestation levels. This highlights the need to complement egg parasitism with predators capable of targeting early nymphal instars immediately after hatching.

Several coccinellid predators have been evaluated for their ability to suppress early developmental stages of *N. viridula*<sup>13,14</sup>. In general, coccinellids exhibited no or negligible predation on eggs and only low levels of predation on early nymphal instars. Other generalists, such as nabids and reduviids, showed moderate to high predation on second-instar nymphs, though typically with no egg predation<sup>13</sup>. The predatory stink bug *Podisus maculiventris* (Say, 1832) (Hemiptera: Pentatomidae) remained the most extensively studied predator of *N. viridula*, capable of preying on all nymphal instars, with highest predation observed on first-instar nymphs and decreasing gradually across later stages<sup>15</sup>. In *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae), larvae displayed limited egg predation<sup>13</sup> but significant predation on early nymphs: third-instar larvae preyed successfully on both first- and second-instar nymphs, while second-instar larvae primarily attacked first-instar nymphs<sup>16</sup>. In contrast, *Geocoris* spp. (Hemiptera: Geocoridae), *Misumenops* spp. (Araneae: Thomisidae), *Oxyopes* spp. (Araneae: Oxyopidae), and various spiders showed minimal or inconsistent predation on *N. viridula*<sup>13</sup>.

While these studies have provided important insights, much of the previous work has concentrated on specific predator-prey combinations, typically addressing individual pest stages or a restricted set of predator taxa. Moreover, little attention has been given to whether commercially available predators, already widely used in European greenhouse systems for controlling whiteflies, aphids, thrips, and lepidopterans, may also contribute to suppressing *N. viridula* nymphs. Key generalists such as *Macrolophus pygmaeus* (Rambur, 1839) (Hemiptera: Miridae), *Orius laevigatus* (Fieber, 1860), *Orius majusculus* (Reuter, 1879) (Hemiptera: Anthocoridae), *Adalia bipunctata* (Linnaeus, 1758), and *Propylea quatuordecimpunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) remain largely unstudied with respect to their interactions with *N. viridula*. For *C. carnea*, previous studies<sup>13,16</sup> have provided detailed insights into its predation potential against early nymphal stages of *N. viridula*. Nevertheless, for many other generalist predators widely used in greenhouse IPM programs, their contribution to *N. viridula* suppression remains insufficiently explored. Understanding whether any of these existing generalist predators can contribute to the suppression of *N. viridula* is highly relevant for strengthening IPM programs. An additional knowledge gap concerns the taxonomic complexity within the *C. carnea* species complex. As shown by Henry et al.<sup>17</sup>, what was traditionally considered *C. carnea* consists of several reproductively isolated, morphologically similar but acoustically distinct sibling species, including *C. carnea* sensu stricto and *Chrysoperla lucasina* (Lacroix, 1912) (Neuroptera: Chrysopidae). These cryptic species may differ in biological traits relevant to biological control, yet their functional roles remain poorly explored. Commercially available populations in Europe may belong to different members of this species complex, but whether such cryptic diversity translates into differences in predation efficiency against *N. viridula* has not been investigated. Therefore, it is particularly relevant to compare representatives of the *C. carnea* species complex in parallel to determine whether species identity influences their potential contribution to the suppression of *N. viridula* nymphs.

The present study aimed to evaluate the predation potential of several commercially available arthropod predators against early developmental stages of *N. viridula*, with particular emphasis on the cryptic green lacewing species *C. lucasina* and *C. carnea*. The research addressed five central questions. First, which predator species and life stages exert the strongest impact on *N. viridula* eggs and/or early nymphal instars under controlled conditions? It was hypothesized that predation would be largely confined to larval stages of *Chrysoperla* spp. and coccinellids, primarily targeting the nymphal instars, while egg predation would remain minimal due to the mechanical protection of the egg chorion and the limited ability of generalist predators to penetrate encapsulated stages<sup>13,14,16</sup>. Second, how does predation vary between immature stages within the best-performing predatory species? It was hypothesized that later developmental stages generally exhibit higher predation rates than earlier ones<sup>16</sup>. This expectation derives from progressive increases in body size, foraging mobility, and morphological traits such as enhanced mouthpart strength or improved sensory capacity, which together facilitate the capture and handling of increasingly mobile or better-defended prey. Third, do cryptic lacewing species differ in predation capacity, and are such differences consistent across larval instars? Although *C. carnea* and *C. lucasina* are cryptic species within the *C. carnea* species complex, it was hypothesized that they differ functionally as predators due to their distinct ecological histories<sup>17,18</sup> and potentially divergent evolutionary adaptations. Specifically, *C. lucasina* was expected to exhibit somewhat higher predation rate on mobile nymphal stages, potentially reflecting species-specific differences in prey acceptance thresholds, developmental speed, foraging persistence, or prey-handling efficiency that remain concealed without species-level resolution. Fourth, does predation by lacewing larvae remain consistent across short-term exposure periods when prey availability is not a limiting factor, or do predation rates fluctuate, indicating intrinsic predator-driven dynamics? It was hypothesized that any temporal variation in predation rates is attributable primarily to the behavior and physiological state of the predator, such as satiation, foraging persistence, or developmental shifts in motivation, rather than depletion of available prey. Finally, can promising laboratory results be replicated under structurally complex conditions that better reflect commercial crop environments? It was hypothesized that predators showing strong performance in controlled assays would retain their efficacy in these settings, although increased habitat complexity and prey concealment could reduce overall predation efficiency. Collectively, these research questions and hypotheses were formulated

to capture interspecific, intraspecific, ontogenetic, and environmental drivers of predator efficacy and to clarify how cryptic species identity, stage-specific predatory function, and ecological realism intersect to inform precision biological control strategies for *N. viridula* in protected cropping systems.

## Materials and methods

### Insect rearing and colony maintenance

#### *Nezara viridula*

A laboratory colony of *N. viridula* was maintained at the Business Unit Greenhouse Horticulture & Flower Bulbs, Wageningen University & Research (Bleiswijk, the Netherlands), originating from field-collected individuals in the Netherlands and Belgium. Adults were housed in fine-mesh breeding cages (40 × 40 × 60 cm, 0.1 mm mesh; Vermandel, the Netherlands) placed within walk-in climate-controlled rooms at 25.0 ± 1.5 °C, 70 ± 10% RH, and a 16:8 h L: D photoperiod. Flat bean pods (*Phaseolus vulgaris* L.) and sunflower seed kernels (*Helianthus annuus* L.) were provided as food, with water supplied via moistened synthetic cotton. All resources were replenished twice weekly.

Egg masses were collected twice weekly during colony maintenance and daily during experimental periods. To ensure continuous access to all required developmental stages for both colony maintenance and experimental use, they were transferred to plastic boxes (30 × 20 × 13 cm; Curver, the Netherlands) with two to four lateral apertures (each 9 cm in diameter) covered by 80 µm mesh to allow ventilation. Egg masses were placed on folded paper towels inside each box, which also contained buckwheat hulls as habitat substrate. Food was supplied prior to hatching to ensure immediate access for neonates. Nymphs were reared to adulthood within the same boxes, and food was replenished twice weekly. All developmental stages were maintained in climate cabinets (Economic Lux ECL02; Snijders Labs, Tilburg, the Netherlands) at 25.0 ± 1.0 °C, 70 ± 10% RH, and a 16:8 h L: D photoperiod. Upon reaching adulthood, individuals were transferred to the fine-mesh breeding cages within the walk-in climate-controlled rooms.

#### *Chrysoperla lucasina* and *Chrysoperla carnea*

The *C. carnea* colony was established from larvae obtained from Biobest Group N.V. (Westerlo, Belgium), while the *C. lucasina* colony originated from eggs supplied by IF-Tech (Les Ponts-de-Cé, France). Both species were reared separately in plastic boxes (30 × 20 × 13 cm) with two to four lateral apertures (each 9 cm in diameter) covered by 80 µm mesh to allow ventilation, and housed in individual climate cabinets at 25.0 ± 1.0 °C, 70 ± 10% RH, and a 16:8 h L: D photoperiod.

Each box contained buckwheat hulls and crumpled paper towels as habitat substrate and shelter to reduce cannibalism. Larvae were primarily fed *ad libitum* with frozen, sterilized eggs of *Ephesia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae), occasionally supplemented with live pea aphids, *Acyrtosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae); both prey types were supplied by Koppert Biological Systems (Berkel en Rodenrijs, the Netherlands). *Ephesia kuehniella* eggs were presented on the adhesive surface of sticky note strips (Post-it™, 3 M, St. Paul, MN, USA).

Adults were maintained in separate boxes and provided with *Typha angustifolia* pollen (Nutrimite, Biobest Group N. V.) on crumpled paper, along with cotton pads soaked in a 9:1 water-to-sugar solution, placed on Petri dish lids. All food sources were replenished twice weekly. Lids bearing eggs were transferred to new boxes to initiate fresh cultures.

#### *Micromus angulatus*

A colony of the brown lacewing *M. angulatus* was established from adults obtained from Biobest Group N. V. Adults were reared in plastic containers (Ø 22 cm × 26 cm) covered with fine mesh fabric to allow ventilation. Each container held buckwheat hulls and crumpled paper towels as habitat substrate and shelter, and jute fibers were added as oviposition substrates. Larvae were reared separately in plastic boxes (30 × 20 × 13 cm) with two to four lateral apertures (each 9 cm in diameter) covered by 80 µm mesh to allow ventilation. Both adults and larvae were maintained in climate cabinets at 20.0 ± 1.0 °C, 70 ± 10% RH, and a 16:8 h L: D photoperiod.

Adults and larvae were fed *ad libitum* with live pea aphids and frozen, sterilized eggs of *E. kuehniella*, offered on the adhesive surface of sticky note strips. Sugar water was provided via cotton pads soaked in a 9:1 water-to-sugar solution, placed on Petri dish lids. Food and sugar water were replenished twice weekly. Jute fibers bearing eggs were collected twice weekly from the adult rearing containers to initiate new cultures.

#### *Orius laevigatus* and *Orius majusculus*

Adults and nymphs of *O. laevigatus* were obtained from Koppert Biological Systems, and *O. majusculus* from AgroBio (La Albuera, Spain). Both species were reared in plastic jars (Ø 9 cm × 13 cm) with lids containing a 5 cm central aperture covered by 80 µm mesh to allow ventilation. Each jar contained a flat bean pod serving as the oviposition substrate. Buckwheat hulls were added as habitat substrate.

Adults and nymphs were fed *ad libitum* with a 1:1 mixture of frozen, sterilized eggs of *E. kuehniella* and decapsulated cysts of *Artemia franciscana* (Kellogg, 1906) (Anostraca: Artemiidae) (BioArtFeed, BioBee, Sde Eliyahu, Israel), offered on the adhesive surface of sticky note strips. Food and bean pods were replenished twice weekly. Pods bearing predator eggs were transferred to new jars to initiate new cultures. All rearing jars were kept in climate cabinets at 25.0 ± 1.0 °C, 70 ± 10% RH, and a 16:8 h L: D photoperiod.

#### *Macrolophus pygmaeus*

A culture of *M. pygmaeus* was established from adults and nymphs obtained from Koppert Biological Systems and reared in plastic buckets (Ø 22 cm × 26 cm) covered with fine mesh fabric to allow ventilation. Each bucket contained buckwheat hulls as habitat substrate. Predators were fed *ad libitum* with a 1:5 mixture of frozen,

sterilized eggs of *E. kuehniella* and decapsulated cysts of *Artemia* spp. (EntoFood, Koppert Biological Systems), offered on the adhesive surface of sticky note strips. Flat bean pods were added as a water source and oviposition substrate. Food was replenished twice weekly. Cultures were maintained in climate cabinets at  $20.0 \pm 1.0$  °C,  $70 \pm 10\%$  RH, and a 16:8 h L: D photoperiod. Bean pods bearing eggs were collected weekly to initiate new cultures.

#### *Adalia bipunctata*

A colony of *A. bipunctata* was established from larvae obtained from Koppert Biological Systems. Adults and larvae were reared in plastic boxes (30 × 20 × 13 cm) with two to four lateral apertures (each 9 cm in diameter) covered by 80 µm mesh to allow ventilation. Boxes were placed in climate cabinets at  $25.0 \pm 1.0$  °C,  $70 \pm 10\%$  RH, and a 16:8 h L: D photoperiod.

Individuals were fed *ad libitum* with live pea aphids and frozen, sterilized eggs of *E. kuehniella*, and offered on the adhesive surface of sticky note strips. Buckwheat hulls and crumpled paper towels were provided as habitat substrate, shelter, and oviposition sites to reduce cannibalism. Food was replenished twice weekly. Crumpled towels and box lids bearing eggs were collected regularly to initiate new cultures.

#### *Propylea quatuordecimpunctata*

A colony of *P. quatuordecimpunctata* was established by combining two distinct adult populations obtained from Biobest Group and Andermatt Nederland (formerly Entocare, Wageningen, the Netherlands). The colony was reared in plastic boxes (30 × 20 × 13 cm) with two to four lateral apertures (each 9 cm in diameter) covered by 80 µm mesh to allow ventilation. Boxes were placed in climate cabinets at  $25.0 \pm 1.0$  °C,  $70 \pm 10\%$  RH, and a 16:8 h L: D photoperiod.

Adults and larvae were fed with green peach aphids (*Myzus persicae* Sulzer, 1776) (Hemiptera: Aphididae) on sweet pepper leaves (*C. annuum* cv. Maranello), along with a mixture of alternative food composed of 2.5 g bee pollen, 2.5 g *A. franciscana* cysts, 2.5 g *E. kuehniella* eggs, 12.5 g honey, 2.5 g agar, and 50 mL distilled water. Corrugated cardboard strips were provided as oviposition sites and shelter to reduce cannibalism. Food was replenished twice weekly. Cardboard strips bearing eggs were collected regularly to initiate new cultures.

#### Predation bioassays with early life stages of *Nezara viridula*

Predation bioassays were conducted using eggs, first instar (N1), and second instar (N2) nymphs of *N. viridula* as prey, exposed to eight predator species at various life stages (Table 1). Assays were performed under controlled laboratory conditions for a duration of 48 h. Experimental arenas consisted of plastic boxes (18 × 13 × 7 cm) with rectangular vents (10 × 7 cm) on the lid, covered with 80 µm fine mesh. Boxes were single-use, while lids were washed with soap, dried, and reused. Each arena contained a 5 cm piece of flat bean pod, with both cut ends sealed using paraffin wax to delay mold growth and to exclude access by *N. viridula* nymphs or predators. As in routine colony maintenance, sunflower seed kernels were provided for nymphs. For egg trials, a 3 × 3 cm piece of sweet pepper leaf was included to mimic natural oviposition substrates. All arenas were maintained in climate cabinets at  $25.0 \pm 1.0$  °C,  $70 \pm 10\%$  RH, and a 16:8 h L: D photoperiod.

A total of 39 predator-prey combinations were tested. Each treatment included a single predator and either 10 N1, 10 N2, or 15 *N. viridula* eggs. An exception was made for *O. laevigatus* against N1 and N2, where approximately five *O. laevigatus* individuals were introduced per replicate instead of a single predator, to compensate for the small size of this species. This corresponded to a predator-to-prey ratio of approximately 1:2 for nymph assays and 1:3 for egg assays. The slightly higher ratio for eggs was used because they are stationary and readily detectable in confined arenas. These ratios were selected to ensure sufficient predation while minimizing overcrowding or interference. Treatments were replicated 5 to 20 times, with higher replication assigned to predators exhibiting promising predation. Controls without predators were included for all prey types, with at least one control per five predator replicates.

Predators were starved for 24 h before testing. To prevent cannibalism and ensure uniform hunger levels, individuals were isolated in plastic cups (Ø 4 cm × 4 cm) with vented lids (80 µm mesh) and moistened cotton for hydration. Nymphs (< 24 h old) were introduced into arenas using a fine paintbrush (1.7 mm), placed on the bean pod, and allowed to acclimate for 1 h. Fresh eggs (< 48 h old) were transferred to sweet pepper leaves using forceps. Predators were introduced using the same paintbrush and placed in a corner of the arena.

Order	Family	Predator species	Life stages
Hemiptera	Miridae	<i>Macrolophus pygmaeus</i>	Adults
	Anthocoridae	<i>Orius majusculus</i>	Adults
		<i>Orius laevigatus</i>	Adults
Neuroptera	Chrysopidae	<i>Chrysoperla carnea</i>	Second and third larval instars
		<i>Chrysoperla lucasina</i>	Second and third larval instars
	Hemerobiidae	<i>Micromus angulatus</i>	Third larval instar and adults
Coleoptera	Coccinellidae	<i>Propylea quatuordecimpunctata</i>	Adults
		<i>Adalia bipunctata</i>	Third and fourth larval instars and adults

**Table 1.** Predator species and life stages tested against the eggs, first- and/or second-instar nymphs of *N. viridula*.

Prey survival was recorded at 24 h and 48 h. Nymphs were assessed visually, while eggs were examined using a flex arm stereomicroscope (Leica MZ9<sub>5</sub>; Leica Microsystems, Wetzlar, Germany) to detect signs of predation, such as collapsed chorions, puncture marks, or emptied contents. Eggs that were not visibly damaged were assumed to hatch, but this was not recorded. After 24 h, prey densities were assessed and replenished with fresh nymphs (24–48 h old) or new eggs to restore initial levels (10 nymphs or 15 eggs per replicate). This ensured consistent prey availability and allowed repeated evaluation of predator feeding at 24 and 48 h. The 48 h design thus allowed assessment of temporal patterns in predation, including potential effects of satiation or behavioral shifts over time.

### Predation of *Nezara viridula* nymphs by *Chrysoperla lucasina* larvae in greenhouse cage trials

To assess predation efficacy under more realistic conditions, the third larval instar (L3) of *C. lucasina* was tested against *N. viridula* nymphs in greenhouse cage trials, as this species had been identified as the most effective predator in laboratory bioassays. Trials were performed in mesh cages (60 × 60 × 90 cm, 0.1 mm mesh; Vermandel, the Netherlands), each enclosing a 2–2.5-month-old sweet pepper plant approximately 80–100 cm in height, bearing flowers and developing fruits to provide a structurally complex arena. The experiment was conducted in a greenhouse maintained at 21.0 ± 3.5 °C, 70 ± 10% RH, and a 16:8 h L:D photoperiod.

Each cage received a total of 20 *N. viridula* nymphs, introduced in a 7:3 ratio of N1 to N2. This ratio was selected to simulate natural post-hatch distributions, where most individuals are still in the N1 stage and only a smaller proportion have molted to N2. Given the increased structural complexity of the arena and the doubled prey density compared to laboratory trials, two L3 larvae of *C. lucasina* were released per cage to ensure sufficient predator pressure.

To standardize prey distribution, each pepper plant (approximately 80–100 cm tall) was visually assessed from the soil surface to the top of the plant. The approximate midpoint of the main stem was estimated by eye and used as a reference. One leaf located above this point and one leaf located below were randomly selected for infestation. The abaxial surface of each leaf was infested with 10 nymphs (7 N1 and 3 N2), totaling 20 prey per plant. Although N1 individuals were placed at separate points on the leaf, they were consistently observed aggregating prior to predator release. All prey were introduced across cages before predators were released to ensure consistency in exposure timing. The use of the abaxial surface mirrors the natural oviposition behavior of *N. viridula*, as females typically lay eggs on the underside of leaves and neonates remain aggregated after hatching.

Predators were starved for 24 h prior to introduction. Two L3 larvae were released onto the main stem, one after the other, approximately 15 cm above the soil using a fine paintbrush. The predator treatment was replicated ten times. Control cages without predators were included to assess background nymph mortality in the absence of predation, with two replicates.

Plants were watered every other day. Prey survival was assessed at 24 h and 48 h using the same criteria as in laboratory assays, with the exception that prey was not replenished after 24 h. This allowed for evaluation of cumulative predation over 48 h under fixed prey availability.

### Statistical analyses

All statistical analyses were performed in R version 4.4.2<sup>19</sup> using the packages MASS<sup>20</sup>, emmeans<sup>21</sup>, dplyr<sup>22</sup>, glmmTMB<sup>23</sup>, car<sup>24</sup>, DHARMa<sup>25</sup>, and ggplot2<sup>26</sup>. All visualizations were generated using ggplot2. Approximately 1% of predators either died or molted during the 48 h experimental period and were excluded from analyses.

For laboratory assays, generalized linear models (GLMs) with Poisson error distribution and log link function were fitted to analyze the number of intact eggs or surviving *N. viridula* nymphs at each prey stage (eggs, N1, N2) and time point (24 h and 48 h). Predator treatment (species and life stage) was included as a fixed effect. Dispersion was evaluated using the ratio of residual deviance to degrees of freedom. When over- or underdispersion was detected, models were refitted using negative binomial or quasi-Poisson distributions to ensure valid inference. Model fit was assessed by comparing full models to intercept-only null models using chi-squared tests on residual deviance. When treatment effects were significant ( $p < 0.05$ ), pairwise comparisons between treatments and controls were performed using estimated marginal means (emmeans package) with Tukey-adjusted  $p$ -values.

To examine temporal changes in predation by *C. carnea* and *C. lucasina*, survival of *N. viridula* N1 and N2 was analyzed separately across two time points (24 h and 48 h). For each instar, GLMs with a Poisson error distribution and log link function were fitted, with predator treatment (species and developmental stage), time point, and their interaction as fixed effects. Overdispersion in the N1 model was addressed by refitting with a negative binomial distribution; underdispersion in the N2 model was corrected using a quasi-Poisson distribution. Model terms were added sequentially, and significance was evaluated using chi-squared tests on residual deviance. Pairwise comparisons between time points (24 h vs. 48 h) were performed within each predator treatment using the same procedure as above.

For intraspecific differences in predation efficacy between chrysopid larval stages, separate models were fitted for *C. carnea* and *C. lucasina*, comparing the effects of L2 versus L3 on *N. viridula* nymphal survival. For each predator species and *N. viridula* nymphal instar (N1 and N2), GLMs with a Poisson error distribution and log link function were initially fitted, with predator life stage (L2, L3) included as a fixed effect. Overdispersion in the N1 models was addressed by refitting with a negative binomial distribution. For N2, underdispersion in the *C. carnea* model and mild overdispersion in the *C. lucasina* model were corrected using quasi-Poisson distributions. Model fit, and significance testing followed the same procedures as described before, with pairwise comparisons conducted within species.

To assess interspecific differences in predation efficacy between *C. carnea* and *C. lucasina* across larval stages, separate GLMs with a Poisson error distribution and log link function were fitted for each *N. viridula* nymphal

instar (N1 and N2). Each model included predator species, life stage (L2, L3), and their interaction as fixed effects. For N1, overdispersion was addressed by refitting the model with a negative binomial distribution. For N2, underdispersion was corrected using a quasi-Poisson distribution. Model terms were added sequentially and tested using chi-squared tests on residual deviance. Pairwise comparisons between predator species were performed within each life stage.

For prey-stage-specific susceptibility to predation by *C. lucasina*, survival of *N. viridula* nymphs was compared between N1 and N2 under exposure to L2 and L3 predator stages. A GLM with a Poisson error distribution and log link function was initially fitted, with predator life stage (L2, L3), prey stage (N1, N2), and their interaction as fixed effects. Overdispersion was addressed by refitting with a negative binomial distribution. Pairwise comparisons between prey stages were performed within each predator life stage.

To assess predation in greenhouse cage trials without distinguishing between *N. viridula* instars, the total number of surviving nymphs was analyzed using a generalized linear mixed model (GLMM) with a Poisson error distribution and log link function, fitted with the glmmTMB package. Fixed effects included treatment (predator vs. control), time point (24 h, 48 h), and their interaction; replicate was included as a random intercept to account for repeated observations. Type II Wald chi-square tests were used to assess the significance of fixed effects. Pairwise comparisons between treatment combinations were performed using estimated marginal means with Tukey-adjusted *p*-values. Model assumptions were validated using the DHARMA residual diagnostics.

To further evaluate instar-specific survival at 48 h in greenhouse cage trials, separate models were fitted for N1 and N2 *N. viridula* nymphs. For each instar, the number of surviving individuals was analyzed using GLMMs with a Poisson error distribution and log link function, with treatment (predator vs. control) included as a fixed effect and replicate as a random intercept. To assess susceptibility differences between *N. viridula* nymphal instars within the *C. lucasina* L3 treatment, the number of surviving and dead individuals per replicate was analyzed using a GLMM with a binomial error distribution and logit link function, with prey stage (N1 vs. N2) as a fixed effect and replicate as a random intercept. Type II Wald chi-square tests were used to evaluate the significance of fixed effects. Pairwise comparisons between treatments or prey stages were performed using estimated marginal means with Tukey-adjusted *p*-values. Model assumptions were validated using the DHARMA diagnostics.

## Results

### Ontogenetic variation in predation vulnerability of early-stage *Nezara viridula*

#### Egg stage

The number of intact *N. viridula* eggs differed significantly across predator treatments at both 24 and 48 h (GLM, 24 h:  $\chi^2 = 40.689$ ,  $df = 12$ ,  $p < 0.001$ ; 48 h:  $\chi^2 = 11.919$ ,  $df = 12$ ,  $p < 0.001$ ).

At 24 h, egg survival was significantly reduced in the *C. lucasina* L2 (Tukey's test,  $z = -3.596$ ,  $p = 0.02$ ) and L3 ( $z = -6.713$ ,  $p < 0.001$ ) treatments, with mean survival of  $74.7 \pm 9.57\%$  and  $60.0 \pm 6.67\%$ , respectively, compared to  $100 \pm 0\%$  in the control (Fig. 1a).

At 48 h, both *C. lucasina* L2 and L3 again significantly reduced egg survival compared to the control (Tukey's test, *C. lucasina* L2 vs. control:  $z = -4.151$ ,  $p = 0.002$ ; *C. lucasina* L3 vs. control:  $z = -3.592$ ,  $p = 0.02$ ), with mean survival of  $77.3 \pm 8.02\%$  and  $82.7 \pm 5.44\%$ , respectively, versus  $100 \pm 0\%$  in the control (Fig. 1b). These values reflect mortality during the second 24 h interval (i.e. 24–48 h), assessed independently from the first (0–24 h). No other predator treatment differed significantly from the control at either time point.

#### First-instar nymphs

The number of surviving *N. viridula* first-instar nymphs differed significantly across predator treatments at both 24 and 48 h (GLM, 24 h:  $\chi^2 = 283.50$ ,  $df = 13$ ,  $p < 0.001$ ; 48 h:  $\chi^2 = 297.45$ ,  $df = 13$ ,  $p < 0.001$ ).

At 24 h, survival was significantly reduced relative to the control under exposure to *C. carnea* L2 (Tukey's test,  $z = -4.597$ ,  $p < 0.001$ ), *C. carnea* L3 ( $z = -9.255$ ,  $p < 0.001$ ), *C. lucasina* L2 ( $z = -6.369$ ,  $p < 0.001$ ), and *C. lucasina* L3 ( $z = -10.916$ ,  $p < 0.001$ ), with mean survival of  $55.6 \pm 8.68\%$ ,  $29.3 \pm 6.28\%$ ,  $42 \pm 10.6\%$ , and  $8.12 \pm 2.28\%$ , respectively, compared to  $97.9 \pm 1.04\%$  in the control (Fig. 2a).

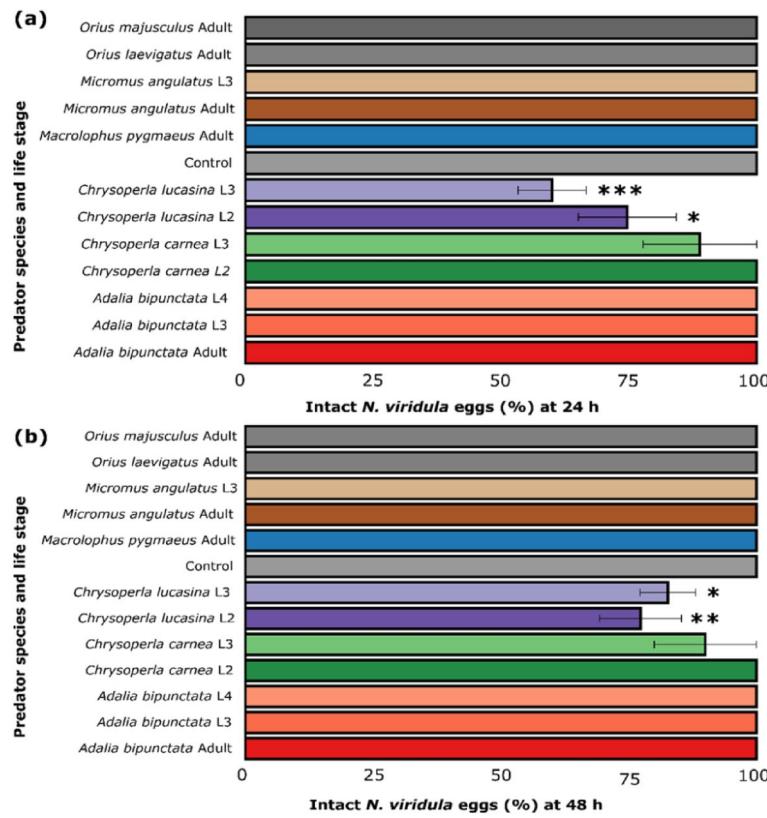
At 48 h, *C. carnea* L2 (Tukey's test,  $z = -3.526$ ,  $p = 0.029$ ), *C. carnea* L3 ( $z = -10.160$ ,  $p < 0.001$ ), *C. lucasina* L2 ( $z = -9.541$ ,  $p < 0.001$ ), and *C. lucasina* L3 ( $z = -12.557$ ,  $p < 0.001$ ) again yielded significantly lower survival relative to the control, with mean survival of  $70 \pm 5.53\%$ ,  $32.7 \pm 5.11\%$ ,  $23 \pm 7\%$ , and  $8.75 \pm 2.72\%$ , respectively, compared to  $99 \pm 0.576\%$  in the control (Fig. 2b). No other predator treatment differed significantly from the control at either time point.

#### Second-instar nymphs

The number of surviving *N. viridula* second-instar nymphs differed significantly across predator treatments at both 24 and 48 h (GLM, 24 h:  $\chi^2 = 147.56$ ,  $df = 11$ ,  $p < 0.001$ ; 48 h:  $\chi^2 = 106.65$ ,  $df = 11$ ,  $p < 0.001$ ).

At 24 h, significant reductions in N2 survival relative to the control were seen in *C. lucasina* L3 (Tukey's test,  $z = -17.465$ ,  $p < 0.001$ ), *C. carnea* L3 ( $z = -10.290$ ,  $p < 0.001$ ), *C. lucasina* L2 ( $z = -8.217$ ,  $p < 0.001$ ), and *C. carnea* L2 ( $z = -4.009$ ,  $p = 0.004$ ) treatments, with mean survival of  $20 \pm 4.06\%$ ,  $26 \pm 2.45\%$ ,  $57 \pm 1.53\%$ , and  $80.7 \pm 3.05\%$ , respectively, compared to  $100 \pm 0\%$  in the control (Fig. 3a).

At 48 h, the same four treatments remained significantly lower than the control: *C. carnea* L3 (Tukey's test,  $z = -8.689$ ,  $p < 0.001$ ), *C. lucasina* L3 ( $z = -12.941$ ,  $p < 0.001$ ), *C. lucasina* L2 ( $z = -9.614$ ,  $p < 0.001$ ), and *C. carnea* L2 ( $z = -3.632$ ,  $p = 0.015$ ), with mean survival of  $32 \pm 6.63\%$ ,  $35.7 \pm 5.81\%$ ,  $45 \pm 1.67\%$ , and  $80.7 \pm 3.70\%$ , respectively, compared to  $100 \pm 0\%$  in the control (Fig. 3b). No other predator treatment differed significantly from the control at either time point.



**Fig. 1.** Survival of *Nezara viridula* eggs after exposure to different predator species and life stages for 24 h (a) and 48 h (b). Bars represent the mean ( $\pm$  SE) percentage of *N. viridula* eggs that remained intact following exposure to the different predator treatments. The y-axis lists the predator species and their life stages: adults, second instar larvae (L2), third instar larvae (L3), and fourth instar larvae (L4). The x-axis indicates the percentage of intact *N. viridula* eggs recorded at each time point (24–48 h). Prey were replenished after 24 h. Asterisks denote significant differences from the control based on Tukey's test:  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*). Replicates per treatment:  $n = 19$  (control), 5 (*A. bipunctata* L3/L4/Adult, *O. laevigatus* Adult, *O. majusculus* Adult, *M. angulatus* Adult), 6 (*C. carnea* L3), 9 (*C. carnea* L2, *M. pygmaeus* Adult), 10 (*C. lucasina* L2), 15 (*C. lucasina* L3), 2 (*M. angulatus* L3). The low number of replicates for *M. angulatus* L3 was due to predator mortality during the assay; only replicates with live predators were included in the analysis.

### Predation stability over time in the larvae of *Chrysoperla* species

#### First-instar nymphs

Survival of *N. viridula* first-instar nymphs differed significantly among predator treatments (GLM,  $\chi^2 = 92.387$ ,  $df = 3$ ,  $p < 0.001$ ), while neither the main effect of time point ( $\chi^2 = 0.012$ ,  $df = 1$ ,  $p = 0.911$ ) nor the interaction between treatment and time point ( $\chi^2 = 5.387$ ,  $df = 3$ ,  $p = 0.146$ ) was significant.

A significant difference between 24 h and 48 h was observed only for *C. lucasina* L2 (Tukey's test,  $z = 2.033$ ,  $p = 0.042$ ), with survival declining from  $42 \pm 10.60\%$  to  $23 \pm 7\%$  (Fig. 4a). No significant temporal differences were found for *C. carnea* L2 ( $z = -0.955$ ,  $p = 0.340$ ), *C. carnea* L3 ( $z = -0.452$ ,  $p = 0.652$ ), or *C. lucasina* L3 ( $z = -0.185$ ,  $p = 0.854$ ).

#### Second-instar nymphs

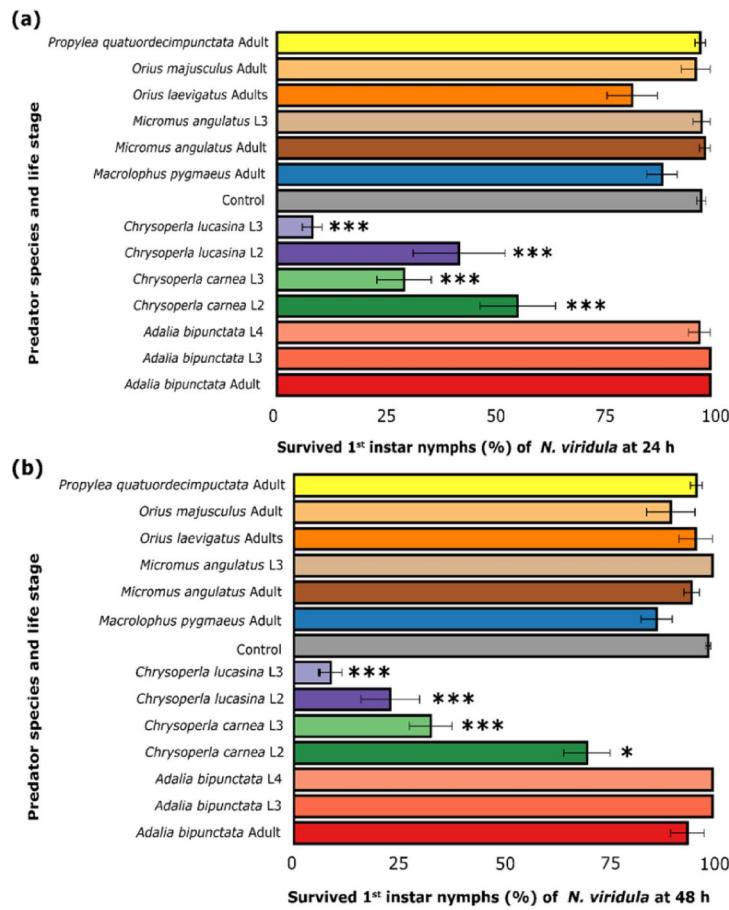
Survival of *N. viridula* second-instar nymphs differed significantly across predator treatments (GLM,  $\chi^2 = 87.597$ ,  $df = 3$ ,  $p < 0.001$ ), and the interaction between treatment and time point was also significant ( $\chi^2 = 7.628$ ,  $df = 3$ ,  $p = 0.003$ ). The main effect of time point was not significant ( $\chi^2 = 0.389$ ,  $df = 1$ ,  $p = 0.393$ ).

A significant difference between 24 h and 48 h was detected only for *C. lucasina* L3 (Tukey's test,  $z = -3.367$ ,  $p < 0.001$ ), with survival increasing from  $20 \pm 4.06\%$  to  $35.7 \pm 5.81\%$  (Fig. 4b). No significant temporal differences were found for *C. carnea* L2 ( $z = 0.000$ ,  $p = 1.000$ ), *C. carnea* L3 ( $z = -0.762$ ,  $p = 0.446$ ), or *C. lucasina* L2 ( $z = 1.625$ ,  $p = 0.104$ ).

### Intraspecific differences in predation efficacy between larval instars of *Chrysoperla* species

#### First-instar nymphs

Survival of *N. viridula* first-instar nymphs differed significantly between L2 and L3 larvae of both *C. carnea* and *C. lucasina* (GLM, *C. carnea*:  $\chi^2 = 22.867$ ,  $df = 1$ ,  $p < 0.001$ ; *C. lucasina*:  $\chi^2 = 23.586$ ,  $df = 1$ ,  $p < 0.001$ ). In *C. carnea*,  $62.8 \pm 5.29\%$  of *N. viridula* first-instar nymphs survived under L2 exposure, compared to  $31 \pm 3.99\%$  under L3



**Fig. 2.** Survival of first-instar *Nezara viridula* nymphs after exposure to different predator species and life stages for 24 h (a) and 48 h (b). Bars represent the mean ( $\pm$  SE) percentage of *N. viridula* first instars that remained alive following exposure to the different predator treatments. The y-axis lists the predator species and their life stages: adults, second instar larvae (L2), third instar larvae (L3), and fourth instar larvae (L4). The x-axis indicates the percentage of surviving *N. viridula* first instar nymphs recorded at each time point (24–48 h). Prey were replenished after 24 h. Asterisks denote significant differences from the control based on Tukey's test:  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*). Replicates per treatment:  $n = 29$  (control), 8 (*A. bipunctata* L3), 4 (*A. bipunctata* L4), 5 (*A. bipunctata* Adult), *O. laevigatus* Adults, *M. angulatus* L3), 9 (*C. carnea* L2, *M. pygmaeus* Adult), 10 (*C. lucasina* L2), 15 (*C. carnea* L3), 16 (*C. lucasina* L3), 8 (*M. angulatus* Adult), 3 (*O. majusculus* Adult), 13 (*P. quatuordecimpunctata* Adult). The low number of replicates for *O. majusculus* Adults was due to predator mortality; only replicates with live predators were included in the analysis.

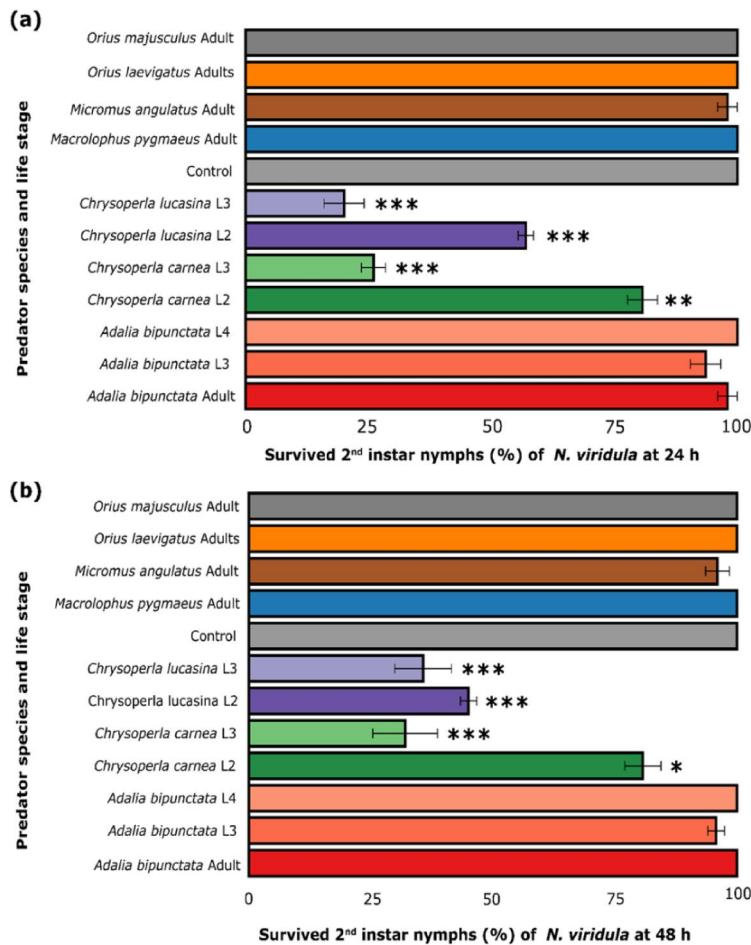
(Tukey's test,  $z = 4.772$ ,  $p < 0.001$ ). In *C. lucasina*,  $32.5 \pm 6.56\%$  of *N. viridula* first-instar nymphs survived under L2 exposure, compared to  $8.4 \pm 1.75\%$  under L3 ( $z = 4.695$ ,  $p < 0.001$ ) (Fig. 5a).

#### Second-instar nymphs

Survival of *N. viridula* second-instar nymphs also varied significantly between L2 and L3 larvae of both *C. carnea* and *C. lucasina* (GLM, *C. carnea*:  $\chi^2 = 34.803$ ,  $df = 1$ ,  $p < 0.001$ ; *C. lucasina*:  $\chi^2 = 16.356$ ,  $df = 1$ ,  $p < 0.001$ ). In *C. carnea*,  $80.7 \pm 2.35\%$  of *N. viridula* second-instar nymphs survived under L2 exposure, compared to  $29 \pm 3.48\%$  under L3 (Tukey's test,  $z = 10.411$ ,  $p < 0.001$ ). In *C. lucasina*,  $51 \pm 1.76\%$  of second-instar nymphs survived under L2 exposure, compared to  $27.9 \pm 3.79\%$  under L3 ( $z = 4.241$ ,  $p < 0.001$ ) (Fig. 5b).

#### Interspecific differences in larval-stage predation by *Chrysoperla* species against early instars of *Nezara viridula*

Survival of *N. viridula* first and second instar nymphs was significantly influenced by predator species (*C. carnea* vs. *C. lucasina*), larval stage (L2 vs. L3), and their interaction. For first-instar *N. viridula* nymphs, significant effects were detected for predator species (GLM,  $\chi^2 = 39.076$ ,  $df = 1$ ,  $p < 0.001$ ), larval stage ( $\chi^2 = 42.928$ ,  $df = 1$ ,  $p < 0.001$ ), and their interaction ( $\chi^2 = 4.452$ ,  $df = 1$ ,  $p = 0.035$ ). Similarly, for second instar *N. viridula* nymphs, survival was affected by predator species (GLM,  $\chi^2 = 36.439$ ,  $df = 1$ ,  $p < 0.001$ ), larval stage ( $\chi^2 = 48.197$ ,  $df = 1$ ,  $p < 0.001$ ), and their interaction ( $\chi^2 = 2.962$ ,  $df = 1$ ,  $p = 0.028$ ).



**Fig. 3.** Survival of second-instar *Nezara viridula* nymphs after exposure to different predator species and life stages for 24 h (a) and 48 h (b). Bars represent the mean ( $\pm$  SE) percentage of *N. viridula* second instars that remained alive following exposure to the different predator treatments. The y-axis lists the predator species and their life stages: adults, second instar larvae (L2), third instar larvae (L3), and fourth instar larvae (L4). The x-axis indicates the percentage of surviving *N. viridula* second instar nymphs recorded at each time point (24–48 h). Prey were replenished after 24 h. Asterisks denote significant differences from the control based on Tukey's test:  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*). Replicates per treatment:  $n = 21$  (control), 14 (*A. bipunctata* L3, *C. carnea* L2, *C. lucasina* L3), 5 (*A. bipunctata* L4/Adult, *C. carnea* L3, *M. angulatus* Adult, *O. majusculus* Adult), 10 (*C. lucasina* L2), 6 (*M. pygmaeus* Adult), 3 (*O. laevigatus* Adults).

At the L2 stage,  $62.8 \pm 5.29\%$  of *N. viridula* first-instar nymphs survived under *C. carnea* compared to  $32.5 \pm 6.56\%$  under *C. lucasina* (Tukey's test,  $z = 4.229$ ,  $p < 0.001$ ). This difference persisted at the L3 stage, with survival decreasing to  $31 \pm 3.99\%$  under *C. carnea* and  $8.4 \pm 1.75\%$  under *C. lucasina* ( $z = 5.953$ ,  $p < 0.001$ ) (Fig. 6a).

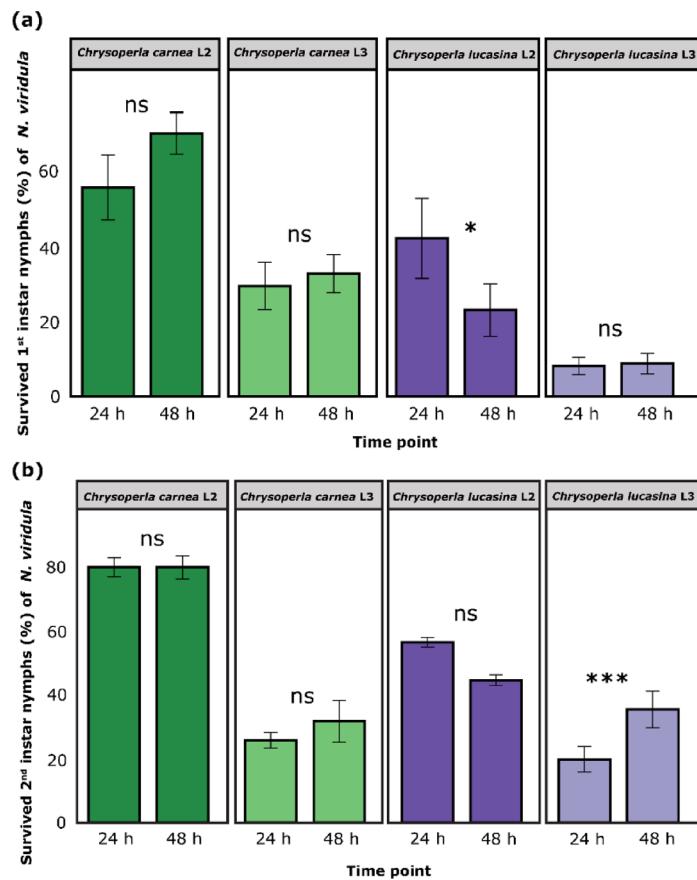
A similar pattern was observed for second-instar nymphs. At L2,  $80.7 \pm 2.35\%$  survived under *C. carnea* compared to  $51 \pm 1.76\%$  under *C. lucasina* (Tukey's test,  $z = 4.915$ ,  $p < 0.001$ ). However, survival at the L3 stage did not differ significantly between species ( $29 \pm 3.48\%$  under *C. carnea* vs.  $27.9 \pm 3.79\%$  under *C. lucasina*;  $z = 0.236$ ,  $p = 0.995$ ) (Fig. 6b).

#### Prey-stage-specific vulnerability to *Chrysoperla lucasina* predation

Survival of *N. viridula* nymphs was significantly influenced by *C. lucasina* larval stage (L2 vs. L3), prey stage (N1 vs. N2), and their interaction (GLM, larval stage:  $\chi^2 = 43.121$ ,  $df = 1$ ,  $p < 0.001$ ; prey stage:  $\chi^2 = 29.914$ ,  $df = 1$ ,  $p < 0.001$ ; interaction:  $\chi^2 = 6.684$ ,  $df = 1$ ,  $p = 0.001$ ). Under L2 exposure,  $32.5 \pm 6.56\%$  of *N. viridula* first instar nymphs survived compared to  $51 \pm 1.76\%$  of second instars (Tukey's test,  $z = -2.545$ ,  $p = 0.053$ ). At the L3 stage, survival was significantly higher in second-instar *N. viridula* nymphs ( $27.9 \pm 3.79\%$ ) than in first instars ( $8.4 \pm 1.75\%$ ) ( $z = -5.141$ ,  $p < 0.001$ ) (Fig. 7).

#### Predation of *Nezara viridula* nymphs by *Chrysoperla lucasina* on pepper plants in greenhouse cage trials

The total number of surviving *N. viridula* nymphs differed significantly across treatments and time points in greenhouse cage trials (GLMM, treatment:  $\chi^2 = 29.286$ ,  $df = 1$ ,  $p < 0.001$ ; time point:  $\chi^2 = 5.1052$ ,  $df = 1$ ,  $p = 0.024$ ;



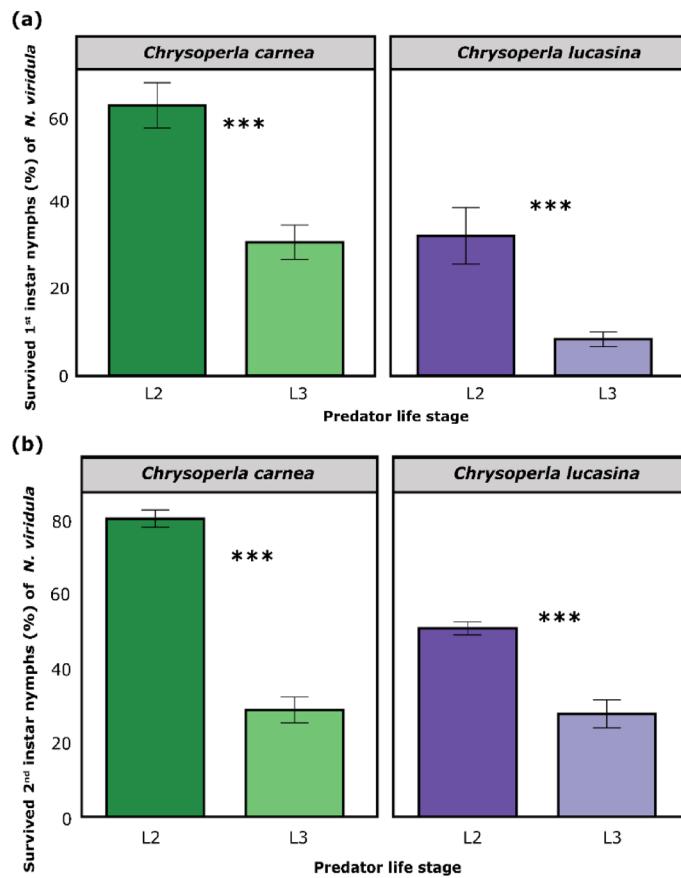
**Fig. 4.** Temporal variation in the survival of *Nezara viridula* first instar (a) and second instar (b) nymphs under predation by *Chrysoperla carnea* and *C. lucasina* larvae. Bars represent the mean ( $\pm$  SE) percentage of *N. viridula* nymphs that remained alive following exposure to second instar (L2) or third instar (L3) larvae of *C. carnea* or *C. lucasina*. The x-axis shows the time points (24 h and 48 h), and the y-axis indicates the percentage of surviving *N. viridula* first or second instar nymphs. Each panel corresponds to a specific predator species and larval stage. Prey were replenished after 24 h. Asterisks denote significant differences between time points based on Tukey's test:  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*); ns = not significant. Replicates per treatment: Fig. 4a –  $n = 9$  (*C. carnea* L2), 15 (*C. carnea* L3), 10 (*C. lucasina* L2), 16 (*C. lucasina* L3); Fig. 4b –  $n = 14$  (*C. carnea* L2), 5 (*C. carnea* L3), 10 (*C. lucasina* L2), 14 (*C. lucasina* L3).

interaction:  $\chi^2 = 2.8583$ ,  $df = 1$ ,  $p = 0.091$ ). At both 24 h and 48 h, *N. viridula* nymphal survival was significantly lower under exposure to *C. lucasina* L3 than in the control (Tukey's test, 24 h:  $z = -3.783$ ,  $p < 0.001$ ; 48 h:  $z = -5.398$ ,  $p < 0.001$ ). Additionally, survival decreased significantly between 24 h and 48 h within the predator treatment ( $z = 2.822$ ,  $p = 0.025$ ) indicating consistent performance (Fig. 8a).

To assess instar-specific susceptibility to predation by *C. lucasina* L3 at 48 h, survival of *N. viridula* nymphs was analyzed separately for N1 and N2. For N1, survival was significantly lower under exposure to *C. lucasina* L3 compared to the control (GLMM,  $\chi^2 = 13.671$ ,  $df = 1$ ,  $p < 0.001$ ). Mean survival was reduced from  $100 \pm 0\%$  in the control to  $30.2 \pm 8.14\%$  in the predator treatment. A similar pattern was observed for N2, with significantly reduced survival under predation ( $\chi^2 = 7.399$ ,  $df = 1$ ,  $p = 0.007$ ), corresponding to a decline from  $100 \pm 0\%$  to  $37 \pm 9.53\%$ . No significant difference in survival was detected between N1 and N2 within the predator treatment ( $\chi^2 = 0.966$ ,  $df = 1$ ,  $p = 0.326$ ), indicating similar susceptibility across instars (Fig. 8b).

## Discussion

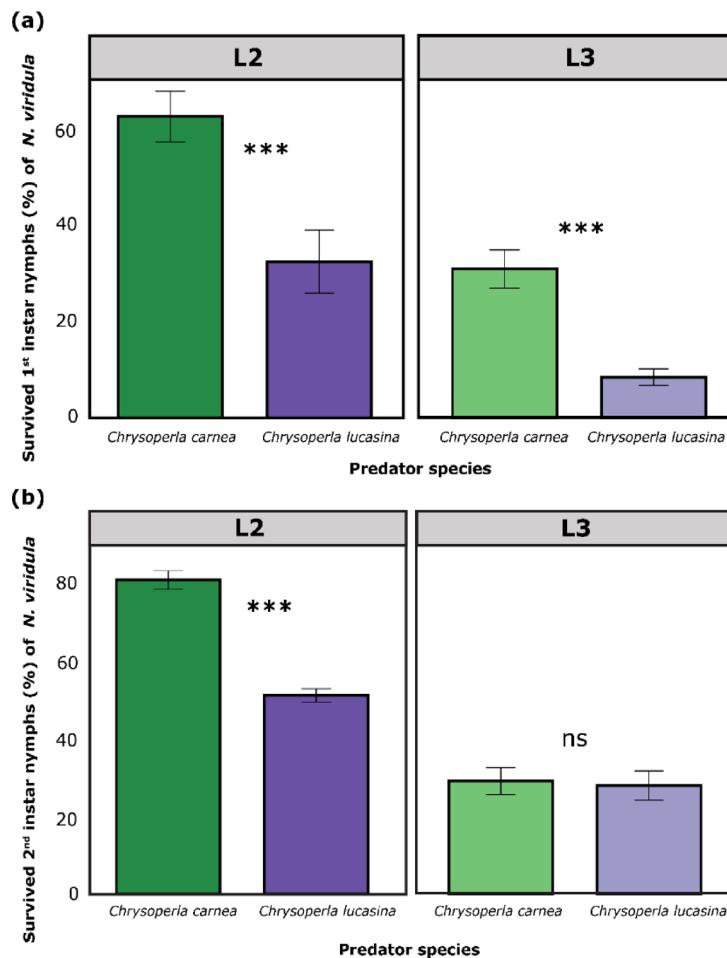
Effective biological control of *N. viridula* depends on targeting the most susceptible stages of its development, typically the eggs and early nymphal instars, where natural enemies can exert the greatest impact. This study presents a comparative evaluation of eight commercially available arthropod predators against *N. viridula* eggs and early nymphal instars (N1 and N2) under controlled laboratory conditions, supplemented by greenhouse cage trials assays to evaluate predator performance in a more structurally complex, crop-relevant context. Predation was largely confined to larvae of *Chrysoperla* spp., with third-instar *C. lucasina* consistently showing the highest mortality across prey stages tested. In nearly every assay, *C. lucasina* outperformed its congener *C. carnea*, revealing important interspecific differences in predatory efficacy. As *C. lucasina* is a cryptic species that can easily be misidentified as *C. carnea*<sup>18</sup>, particularly in commercial or applied contexts, its superior predatory capacity may not have been fully recognized or differentiated in earlier studies and biocontrol efforts.



**Fig. 5.** Intraspecific differences in predation impact of *Chrysoperla carnea* and *C. lucasina* larval stages on *Nezara viridula* first-instar (a) and second-instar (b) nymphs. Bars represent the mean ( $\pm$  SE) percentage of *N. viridula* nymphs that survived following exposure to second (L2) or third (L3) larval instars of *C. carnea* or *C. lucasina*. The x-axis shows the predator larval instar (L2 or L3), and the y-axis indicates the percentage of surviving *N. viridula* nymphs. Each panel corresponds to a predator species (*C. carnea* or *C. lucasina*). Asterisks denote significant differences between larval instars within each predator based on Tukey's test:  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*) $;$  ns = not significant. Replicates per treatment: Fig. 5a –  $n = 9$  (*C. carnea* L2), 15 (*C. carnea* L3), 10 (*C. lucasina* L2), 16 (*C. lucasina* L3); Fig. 5b –  $n = 14$  (*C. carnea* L2), 5 (*C. carnea* L3), 10 (*C. lucasina* L2), 14 (*C. lucasina* L3).

Predation on *N. viridula* eggs was limited or absent for most predator treatments. However, both L2 and L3 of *C. lucasina* consistently reduced egg survival at both 24 h and 48 h. This contrasted with the negligible or absent egg predation observed for *C. carnea*, coccinellids, and other tested predator species. The L3 of *C. carnea* exhibited some feeding activity but did not significantly reduce egg survival relative to the control, aligning with the report by Ehler<sup>13</sup>, which documented low egg attack frequency and predation by *C. carnea*. While *C. carnea* L2 did not attack any eggs in our study, *C. lucasina* L2 intriguingly showed slightly higher egg consumption than its L3. This may reflect differences in developmental state and energetic demand. The L2 individuals used in this study were reared from L1 on *E. kuehniella* eggs, starved for 24 h prior to the assay, and remained in the arena for up to 48 h. All larvae were less than 24 h post-molt into their respective instar at the start of the assay. A few L2 individuals molted to L3 and a few L3 initiated pupation during the 48 h assay; all such replicates were excluded from the analysis. The 24 h starvation period prior to release likely slowed development further. In our laboratory cultures, at  $25 \pm 1$  °C, *C. lucasina* larvae typically required approximately 7–10 days to develop from the start of L1 to the onset of pupation when fed on *E. kuehniella* eggs. Given this developmental trajectory of *C. lucasina* under laboratory conditions, it is likely that many larvae progressively approached the molt to L3 over the course of the assay. This transitional state may have triggered increased feeding effort to meet the metabolic demands associated with larval molting, as observed in holometabolous insects<sup>27,28</sup>. In contrast, some L3 individuals may have been approaching pupation, a phase commonly associated with declining foraging intensity as energy shifts from feeding to preparation for metamorphosis<sup>27,28</sup>. This physiological overlap between late L2 and late L3 could plausibly account for the slightly higher predation by L2.

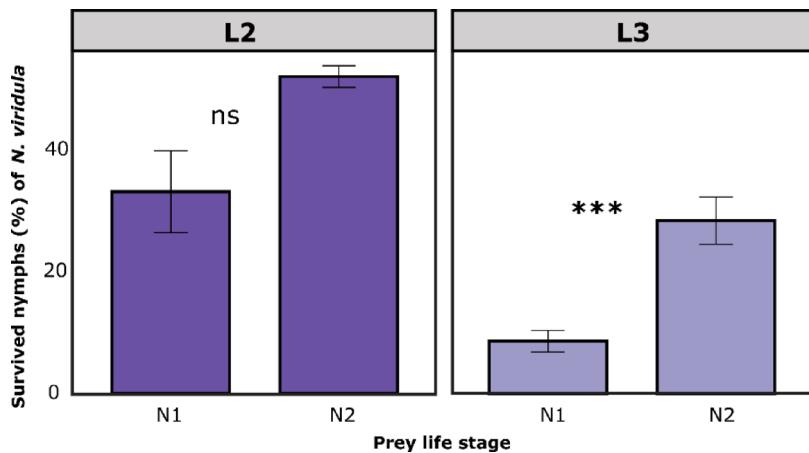
Furthermore, when predation was successful, larvae of *C. lucasina* pierced the chorion and extracted the egg contents entirely, leaving the eggs transparent but structurally intact. A distinct feeding hole was visible on the chorion in all emptied eggs (Fig. 9). In this study, eggs that retained their contents showed no visible signs



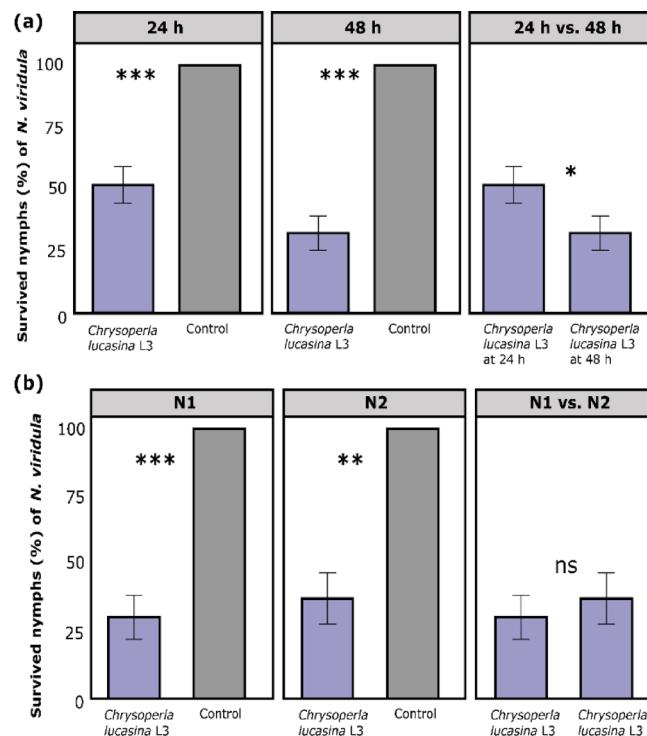
**Fig. 6.** Interspecific comparison of *Chrysoperla carnea* and *C. lucasina* larval stages (L2 and L3) in predation on *Nezara viridula* first instar (a) and second instar (b) nymphs. Bars represent the mean ( $\pm$  SE) percentage of *N. viridula* nymphs that survived following exposure to *C. carnea* or *C. lucasina* larval instars. The x-axis shows predator species at each larval instar (L2 or L3), and the y-axis indicates the percentage of surviving *N. viridula* nymphs. Each panel corresponds to a different larval instar (L2 or L3). Asterisks denote significant differences between predator species within each larval instar based on Tukey's test:  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*); ns = not significant. Replicates per treatment: Fig. 6a –  $n = 9$  (*C. carnea* L2), 15 (*C. carnea* L3), 10 (*C. lucasina* L2), 16 (*C. lucasina* L3); Fig. 6b –  $n = 14$  (*C. carnea* L2), 5 (*C. carnea* L3), 10 (*C. lucasina* L2), 14 (*C. lucasina* L3).

of damage upon stereomicroscope inspection. This feeding pattern was consistently observed in all cases of egg predation.

Behavioral observations during the assays revealed that *C. lucasina* larvae persistently probed egg masses and actively attempted to breach the chorion. Similar probing was observed in *C. carnea* (L2 and L3) and *A. bipunctata* (L3 and L4), but these attempts were brief and never resulted in successful feeding. This suggests that mechanical barriers, rather than chemical defenses, are the primary limitation on egg predation for most species. The inability of most tested species to consume eggs likely reflects the structural integrity of the *N. viridula* egg chorion, underscoring the importance of predator mouthpart structure (mandibular strength in chewing predators) and foraging persistence in determining prey stage susceptibility. Consistent with this, Cottrell and Tillman<sup>14</sup> observed that coccinellid predators failed to access intact *N. viridula* eggs but readily consumed egg contents once the eggs were mechanically crushed, indicating that the rigid chorion presents a substantial mechanical barrier to chewing predators. Moreover, it would be particularly interesting to investigate whether interspecific differences in mandibular strength underlie the observed variation in egg predation. The recently developed “forceX”<sup>29</sup> enables precise and non-destructive bite force measurements even in small insects. Applying such techniques to *C. lucasina* and *C. carnea* species could offer mechanistic insights into their differing capabilities to breach egg chorions. Nevertheless, under no-choice conditions, *C. lucasina* appears capable of breaching this barrier, likely driven by hunger or lack of alternative prey. In realistic settings where more accessible prey such as aphids or early instar nymphs are available, such persistent efforts to prey on eggs are unlikely. Thus, while *C. lucasina* can consume *N. viridula* eggs under constrained conditions, its practical role in suppressing the egg stage is probably limited.



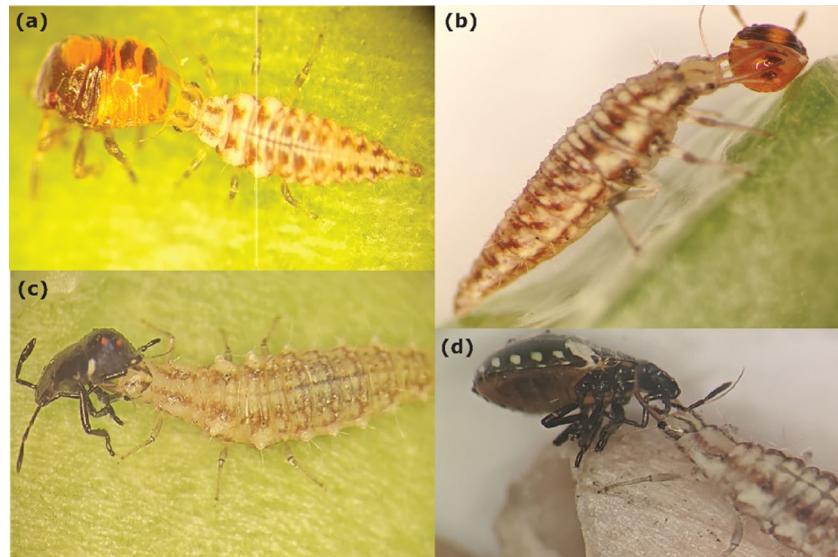
**Fig. 7.** Prey-stage-specific survival of *Nezara viridula* nymphs under predation by *Chrysoperla lucasina* larval instars. Bars represent the mean ( $\pm$  SE) percentage of first instar (N1) and second instar (N2) *N. viridula* nymphs that survived following exposure to second (L2) and third (L3) instar *C. lucasina* larvae. The x-axis denotes the prey stage (N1, N2), and the y-axis shows the percentage of surviving *N. viridula* nymphs. Each panel corresponds to a different *C. lucasina* larval stage (L2 or L3). Asterisks denote significant differences between prey stages within each *C. lucasina* larval stage based on Tukey's test:  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*); ns = not significant. Replicates per treatment: L2 –  $n = 20$  each for N1 and N2; L3 –  $n = 32$  (N1), 28 (N2).



**Fig. 8.** Predation by *Chrysoperla lucasina* third-instar larvae (L3) on *Nezara viridula* nymphs on pepper plants in greenhouse cage trials. **(a)** Mean survival ( $\pm$  SE) of *N. viridula* nymphs at 24 h and 48 h in predator and control treatments. The x-axis indicates the treatment groups for each time point. The right panel compares survival between 24 h and 48 h within the predator treatment. **(b)** Mean survival ( $\pm$  SE) of *N. viridula* nymphal instars (N1 and N2) at 48 h: survival in predator and control treatments (left and middle panels), and comparison between nymphal instars under predator exposure (right panel). The x-axis indicates the treatment or instar comparisons. Asterisks denote significant differences between groups based on Tukey's test:  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*); ns = not significant. Replicates per treatment:  $n = 2$  (control), 9 (*C. lucasina* L3). Each replicate was assessed for both prey stages (N1 and N2) at 24 h and 48 h.



**Fig. 9.** Predation of *Nezara viridula* eggs by the third-instar larvae of *Chrysoperla lucasina*. The cluster shows both predated (transparent) and non-predated eggs (yellow). Predated eggs exhibit complete removal of internal contents while maintaining an intact chorion. The red circle highlights a feeding hole by *C. lucasina* larvae on the chorion.



**Fig. 10.** Predation of *Nezara viridula* nymphal instars by larvae of *Chrysoperla lucasina*. (a) *C. lucasina* L2 feeding on *N. viridula* N1; (b) *C. lucasina* L3 attacking *N. viridula* N1; (c) *C. lucasina* L3 feeding on *N. viridula* N2; (d) *C. lucasina* L3 attacking *N. viridula* N3, demonstrating capacity to subdue larger prey beyond the stages formally assessed.

More substantial biocontrol potential emerged in predation against early instar *N. viridula* nymphs, revealing clear inter- and intraspecific differences in predator efficacy driven by both predator stage and identity. Among all predators tested, *C. lucasina* consistently demonstrated superior performance across instars (Fig. 10a–c). Its L2 larvae already matched or exceeded the efficacy of *C. carnea* L3 against first instars, while *C. carnea* L2 displayed moderate predation on N1 but was largely ineffective against N2. This earlier functional competence of *C. lucasina* suggests species-specific differences in prey-handling capacity, predation thresholds, and foraging motivation, enabling it to exploit mobile prey earlier in development and thereby providing a wider temporal window for intervention in biological control programs.

These findings complement recent results by Berteloot et al.<sup>16</sup>, who reported that predation by *C. carnea* is stage-dependent, with L2 acting primarily against first instars and L3 required for effective predation on second instars. Berteloot et al.<sup>16</sup> excluded egg predation from their study based on previous reports of negligible egg attack by *C. carnea*<sup>13</sup>. In line with these studies, *C. carnea* in our assays did not attack *N. viridula* eggs, while *C. lucasina* readily preyed on eggs, albeit at low levels, reinforcing the functional divergence between these cryptic species across both egg and nymphal stages.

The transition between prey instars further underscores key ecological and mechanistic constraints. First instars, being largely immobile, are highly vulnerable but represent only a brief window for predator exploitation, typically lasting 2 to 5 days under greenhouse conditions at 25 °C<sup>30</sup>. In contrast, second instars exhibit increased mobility, strengthened cuticle, and enhanced escape potential, raising the handling demands for predators. Both

*C. lucasina* and *C. carnea* L3 effectively subdued N2 prey, indicating that by this developmental stage, both predators possess sufficient prey-handling capacity. However, at the L2 stage, only *C. lucasina* maintained strong predation across both instars, while *C. carnea* L2 remained largely ineffective on N2. Interestingly, although not part of the formal bioassays, additional exploratory observations revealed that *C. lucasina* L3 was capable of subduing and consuming third instar *N. viridula* nymphs under laboratory conditions (Fig. 10d), suggesting that its predation potential may extend beyond the developmental stages formally assessed in the present study.

*Micromus angulatus*, another neuropteran predator evaluated in this study, exhibited no predation on any developmental stage of *N. viridula*. Although *M. angulatus* has been described as a promising biological control agent with both larvae and adults preying on aphids<sup>31,32</sup>, its foraging behavior appears strongly aphid-oriented. Pekas et al.<sup>32</sup> emphasized that *M. angulatus* shows limited response to alternative prey such as spider mites, mealybugs, food supplements, or pollen, potentially constraining its ability to establish in the absence of aphids. This dietary specialization likely explains its inactivity against *N. viridula* in the present study. Additionally, after 24 h of pre-assay starvation followed by 48 h exposure to first instar nymphs of *N. viridula* in the experimental arena, approximately 67% of the tested *M. angulatus* L3 larvae were found dead, representing the highest mortality observed across all predator treatments. Consequently, they were excluded from further assays against second instar *N. viridula* nymphs.

The higher *N. viridula* egg and the nymphal predation by *C. lucasina* larvae, particularly in comparison to *C. carnea*, highlights subtle but ecologically relevant interspecific differences within the *Chrysoperla* complex. Despite morphological similarity, the two species significantly diverged in their larval predation capacity. One plausible explanation is evolutionary adaptation to Mediterranean prey dynamics. *Chrysoperla lucasina*, native to Mediterranean regions<sup>17,18</sup>, may have faced selective pressures favoring broader prey acceptance and persistent foraging under conditions of intermittent prey scarcity. Although aphids are typically abundant in these ecosystems during cooler months, their populations often decline sharply during hot, dry summers, creating temporary prey shortages<sup>33,34</sup>. In contrast, stink bug population tend to peak in abundance during late spring through summer<sup>35–37</sup>, with increased oviposition and early nymphal stages during this period. This seasonal overlap likely increased larval encounters with stink bug life stages. Although *N. viridula* originated in East Africa and therefore lacks a shared evolutionary history with European *Chrysoperla* species, its seasonal availability in Mediterranean agroecosystems may still have contributed to behavioral flexibility in local predator populations. These prey, while suboptimal or mechanically defended, might have remained available when aphids were scarce. Such conditions could have selected for generalist traits such as lower prey discrimination thresholds and greater readiness to exploit prey like *N. viridula* eggs and early instar nymphs, regardless of any shared evolutionary history. Additionally, though poorly documented, minor differences in mandible morphology or muscle capacity may confer superior mechanical penetration ability to *C. lucasina* larvae. These functional traits were evident behaviorally: *C. lucasina* larvae, across instars, persistently attempted to breach egg chorions, whereas *C. carnea* showed less persistent effort and lower success. However, this difference may not solely reflect mechanical limitations. If *C. carnea* perceives *N. viridula* eggs as a lower-preference or suboptimal prey, reduced motivation to engage with the eggs could also explain its lower predation levels. Such interspecific differences, although cryptic, carry practical implications for predator performance in biocontrol contexts and highlight the necessity of species-level resolution in selecting biological control agents, especially within cryptic complexes.

Temporal consistency in predation is a critical attribute of effective biological control agents, particularly when pest vulnerability is confined to early developmental stages. In this study, *C. lucasina* and *C. carnea* larvae exhibited distinct temporal dynamics shaped by instar identity and prey stage. On first-instar *N. viridula*, L3 larvae of both species maintained stable predation between 24 and 48 h, while *C. lucasina* L2 increased predation over time, likely reflecting increased energetic demand prior to molting<sup>27,28</sup>. In contrast, *C. lucasina* L3 showed reduced predation over time on second instars, consistent with decreased feeding near pupation. The ability of *C. lucasina* L2 to sustain foraging across time points highlights its overlooked functional role, especially when compared to the slower-onset predation observed in *C. carnea*. While all predators were starved for 24 h prior to testing, potentially elevating initial predation, the limited difference observed between the first and second 24 h intervals in chrysopids suggests that temporal patterns reflect true behavioral dynamics rather than hunger-driven effects. These findings underscore the importance of evaluating predator performance not only by total prey mortality but also by the temporal dynamics of predation.

These patterns were further supported by within-species comparisons. In *C. carnea*, predation increased substantially from L2 to L3, indicating that the ability to handle suboptimal prey and the motivation to do so develop relatively late. In contrast, *C. lucasina* larvae showed a more gradual progression, with L2 already demonstrating strong predatory ability. This supports the view that *C. lucasina* reaches functional maturity earlier, offering a longer effective window for biocontrol. These differences highlight the importance of selecting the appropriate larval stage for augmentative releases, as the timing of predatory development can affect impact across pest cohorts. Prey stage also played a role in shaping predation outcomes. While *C. lucasina* L2 did not show a statistically significant difference in predation between N1 and N2, the trend towards higher efficacy against first instars may reflect a preference or greater ease in handling less mobile prey. However, this pattern became significant only at L3, reinforcing the role of developmental maturity in enhancing prey-stage discrimination and handling efficiency.

The greenhouse cage trials confirmed the strong predatory performance of *C. lucasina* L3. Despite the increased structural complexity and concealment opportunities provided by fruit-bearing sweet pepper plants, *C. lucasina* L3 significantly reduced the survival of *N. viridula* nymphs at both 24 and 48 h. The sustained reduction over time indicates that predation remained active rather than declining due to early satiation, negative post-ingestive feedback, or developmental shifts. Importantly, *C. lucasina* L3 showed comparable predation against both N1 and N2 nymphs, despite differences in prey size, mobility, and use of plant architecture for concealment.

No consistent pattern was observed in the vertical distribution of prey consumption, as nymphs were attacked on both upper and lower plant regions. However, during the early hours of exposure, N1 nymphs were generally targeted before N2s, possibly reflecting a combination of the predator's increased hunger following starvation and the gregarious clustering behavior of newly hatched *N. viridula* nymphs, which may have facilitated initial detection. However, such aggregation does not necessarily enhance overall encounter rates, and dispersed prey may in fact be more likely to be encountered over time due to broader spatial coverage during foraging. That said, once a cluster is located, it can provide a concentrated foraging patch, offering ample prey within a small area. This ability to locate and subdue prey across spatial and behavioral gradients underscores a robust and flexible predatory capacity. The maintenance of foraging activity under these complex conditions further suggests strong behavioral persistence and ecological compatibility with the crop environment. Together, these features position *C. lucasina* L3 as a promising candidate for augmentative biological control of *N. viridula* in greenhouse pepper production systems.

Altogether, this study highlights the importance of predator identity and developmental stage in shaping biological control efficacy against early *N. viridula* instars, offering insights that advance both ecological understanding and applied practice. By disentangling the performance of *C. lucasina* and *C. carnea* across larval instars and prey stages, we address persistent gaps in predator assessment, where differences between cryptic species and stage-specific traits are often overlooked. The consistent superiority of *C. lucasina*, particularly at L2 and L3, not only challenges the widespread reliance on *C. carnea*, but also illustrates the risks of misidentifying morphologically similar taxa when it comes to specific commercial applications. By combining laboratory assays with greenhouse cage trials using fruit-bearing pepper plants, our approach captures a wider range of predator-prey interactions and provides results that are more relevant to real-world cropping systems. Notably, *C. lucasina* L2 exhibited early onset of predatory function and temporal persistence, while L3 maintained strong performance across prey stages and habitat conditions, suggesting a combination of physiological readiness and ecological plasticity. These attributes are especially valuable in protected cropping systems, where pest populations can establish rapidly and opportunities for intervention are brief. The results underscore the importance of aligning predator deployment with the developmental timing of both pests and natural enemies, moving beyond species-level selection to incorporate instar-specific capabilities. Future research should explore how these patterns hold under longer exposure periods, mixed prey environments, and in combination with other biocontrol agents such as egg parasitoids. In particular, interactions between the larvae of *C. lucasina* and *T. basalis* warrant investigation, as intraguild predation on parasitized eggs may not only reduce parasitoid effectiveness but also prevent successful development and emergence, ultimately suppressing parasitoid populations. Such interactions could lead to antagonistic outcomes, reduce overall control efficiency, or destabilize multi-enemy systems. Understanding the direction and magnitude of these effects is essential for designing complementary biocontrol strategies.

## Data availability

The datasets generated during the current study are available from the corresponding author on request.

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## Author contributions

**RRM:** Conceptualization, Methodology, Investigation, Data collection, Data curation, Formal analyses, Writing – original draft, Writing – review and editing, Funding acquisition, Project administration. **RRP:** Investigation, Data collection, Data curation. **AP:** Writing – review and editing, Supervision. **MR:** Writing – review and editing, Supervision. **FLW:** Writing – review and editing, Supervision. **GJM:** Conceptualization, Funding acquisition, Supervision, Project administration, Writing – review and editing.

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## Declarations

### Competing interests

The authors declare no competing interests.

### Additional information

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