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ECOLOGÍA

Life history traits and rearing protocol of *Microvelia pulchella* (Hemiptera: Veliidae): a potential biological control agent and model for behavioral and ecological studies

Rasgos de historia de vida y protocolo de cría de Microvelia pulchella (Hemiptera:Veliidae): un potencial agente de control biológico y modelo para estudios conductuales y ecológicos

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ABSTRACT

Microvelia pulchella, a semiaquatic predatory bug widely distributed in the Neotropics, holds great potential as a biocontrol agent against disease-vector mosquitoes and pests in paddy rice fields. Moreover, insects belonging to the genus *Microvelia* have served as valuable model organisms for ecological and behavioural research. Considering this, our study aimed to establish an enhanced laboratory rearing protocol for *M. pulchella* based on existing methodologies. The protocol encompasses a decision-making flowchart to optimize the rearing process, a standardized method for accurately determining egg and nymph ages, and photographs illustrating all life stages and sexes of the insect. Additionally, we sought to characterize key life history traits of this species. Our detailed rearing procedure involves utilizing different containers tailored to each insect stage and specific requirements. We observed an average development time of 20.6 days from egg to adult at a temperature of 25°C (room temperature). Interestingly, male adults reared in the laboratory exhibited smaller sizes compared to their field-collected counterparts, while females displayed similar sizes across conditions. Female *M. pulchella* demonstrated an average egg production of 211.77 eggs, with the highest fecundity occurring within the first five weeks. Fertility followed a similar pattern, peaking during this period. In terms of longevity, females exhibited an average survival time of 74 days, whereas males lived for approximately 91 days. *M. pulchella* proves to be a convenient model organism for conducting non-invasive experiments, given the multitude of informative traits that can be measured. Additionally, the rearing procedure is cost-effective, straightforward, and requires minimal space.

Keywords: bioassay, broad-shouldered water strider, life cycle, model organism, predatory insect, semiaquatic bug.

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RESUMEN

Microvelia pulchella, un chinche semiacuático depredador presente en el Neotrópico, es prometedor como control biológico de mosquitos vectores y plagas en campos de arroz. Los insectos del género *Microvelia* son importantes organismos modelo para la investigación en ecología y comportamiento. Considerando esto, nuestro estudio tuvo como objetivo establecer un protocolo mejorado de cría en laboratorio para *M. pulchella* basado en metodologías existentes. El protocolo contiene un diagrama de decisiones para mejorar la cría, un método estándar para precisar edades de huevos y ninfas, y fotografías ilustrativas de todas las etapas de vida y sexos del insecto. Nuestro procedimiento de cría detallado emplea recipientes adaptados a cada etapa del insecto y sus requisitos específicos. Observamos un tiempo promedio de desarrollo de 20.6 días desde el huevo hasta el adulto a una temperatura de 25°C (temperatura ambiente). Curiosamente, los machos criados en laboratorio fueron más pequeños que los del campo, mientras que las hembras mantuvieron tamaños constantes en todas las condiciones. Las hembras de *M. pulchella* mostraron una producción promedio de huevos de 211.77, la fecundidad máxima se evidenció durante las primeras cinco semanas. La fertilidad siguió un patrón similar, alcanzando su punto máximo durante este período. En cuanto a la longevidad, las hembras tuvieron un tiempo promedio de supervivencia de 74 días, mientras que los machos vivieron aproximadamente 91 días. Considerando estos resultados, *M. pulchella* es un organismo modelo adecuado para experimentos no invasivos con rasgos informativos medibles. Además, el procedimiento de cría es eficiente, sencillo y requiere poco espacio.

Palabras clave: bioensayo, chinche semiacuático, ciclo de vida, insecto depredador, insecto semiacuático, organismo modelo.

INTRODUCTION

The broad-shouldered water strider *Microvelia pulchella* Westwood, 1834 (Hemiptera: Heteroptera: Gerromorpha: Veliidae) is the most widely distributed species in the Neotropics within the genus *Microvelia* (Moreira *et al.* 2010). This semiaquatic-bug species has been recorded from Canada to Argentina and the Caribbean islands, with altitudinal records ranging from sea level up to 2200 m (Aristizábal-García 2017). Insects in the genus *Microvelia* are characterized by their small size (1.0 – 3.4 mm) (Andersen and Weir 2003). *Microvelia pulchella* can be differentiated from its congeners by its small size (1.25 – 2.25 mm), the curved tibiae in males, and females with a wide groove between the front coxae (in which the rostrum is harbored), this groove having gradually sloping inner edges, which diverge in the posterior part (Epler 2006). According to Taylor and McPherson (1999), *M. pulchella* has four nymphal stages, determined based on mesotibiae measurements in field-collected and laboratory-reared individuals.

Upon reaching maturity, differentiation of adults and late instar nymphs can be problematic as *M. pulchella* individuals exhibit wing polymorphism (Taylor and McPherson 1999, Aristizábal-García 2017), which may be related to environmental cues as observed by Margalef (1983). Seemingly, the apterous and brachypterous forms are dominant in permanent waters, while the winged forms are more common in less persistent water bodies, as they would need to migrate during drought. On the other hand, Aristizábal-García (Aristizábal-García 2017) pointed out that wing polymorphism in this species may appear as a biogeographical feature, where apterous forms predominate in the tropics.

Microvelia pulchella inhabits the water surface in a wide variety of lentic and lotic environments (Taylor and McPherson 1999). Both nymphs and adult forms of this species are predators and scavengers, feeding mainly on arthropods trapped in the water surface film or on organisms associated with aquatic vegetation or crops (Van Driesche *et al.* 2008, Ditrich and Papáček 2010, Aristizábal-García 2017). Overall, the genus *Microvelia* is regarded as a potential biological control agent, as it is known to feed on

larvae of disease-vector mosquitoes like *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae) (Miura and Takahashi 1988, Ohba *et al.* 2011), but also on paddy rice pests like *Nilaparvata lugens* (Stål, 1854) (Hemiptera: Delphacidae) (Nakasuji and Dyck 1984, Sonoda *et al.* 1992), *Nephotettix nigropictus* (Stål, 1870), *N. virescens* (Distant, 1908) (Hemiptera: Cicadellidae), and *Sogatella furcifera* (Horváth, 1899) (Hemiptera: Delphacidae) (Heong *et al.* 1992, Chen *et al.* 2005, Xiao and Tang 2007).

In addition to being a potential biological control agent (by regulating populations of pest species in the paddy rice agroecosystem), *M. pulchella* is a potential model species for laboratory and field experiments, according to the main characteristics of a model organism (Ankeny and Leonelli 2011, Russell *et al.* 2017). For instance, different species within the genus *Microvelia* have been used for ethological studies (Ditrich and Boukal 2016, Toubiana *et al.* 2021), some of them focusing on mating (Travers and Sih 1991, Toubiana and Khila 2019, Matsushima *et al.* 2021, Matsushima and Yokoi 2022), and predatory behaviour (Nakasuji and Dyck 1984, Miura and Takahashi 1988, Jackson and Walls 1998, Ohba *et al.* 2011). Furthermore, *Microvelia* has served as a good model for population dynamics studies (Heong *et al.* 1992, Ditrich and Papáček 2010), phenotypic plasticity (Muraji and Nakasuji 1988), and voltinism (Taylor and McPherson 1999).

There is scant information about the methods for rearing *Microvelia* in the literature. In the studies found, the descriptions of procedures and materials are brief, and in some cases, insufficient to infer the success of the protocol. In those previous studies, insects were reared in groups (Taylor and McPherson 1999) or individually (Ditrich and Papáček 2010). Also, the authors have used different containers for housing insects, including enamel pans (Miura and Takahashi 1988), aquariums (Ditrich and Papáček 2010), plastic jars, and Petri dishes (Nakasuji and Dyck 1984, Sonoda *et al.* 1992, Taylor and McPherson 1999, 2003, Xiao and Tang 2007, Matsushima *et al.* 2021). Similarly, the substrate used for oviposition and as a resting site included filter paper (Sonoda *et al.* 1992, Matsushima *et al.* 2021), cardboard paper strips, plastic disks (Taylor and McPherson 1999, 2003), rice leaf floats (Nakasuji and Dyck 1984), plastic foam (Miura and Takahashi 1988) and polystyrene pieces (Ditrich and Papáček 2010). Regarding the diet, some researchers used *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) (Sonoda *et al.* 1992, Taylor and McPherson 1999), *S. furcifera* (Xiao and Tang 2007), *Lucilia cuprina* (Wiedemann, 1830) (Diptera: Calliphoridae) (Matsushima *et al.* 2021), or a combination of prey (Sonoda *et al.* 1992).

The main objective of this study was to establish a comprehensive protocol, aimed at enhancing existing rearing methods (Nakasuji and Dyck 1984; Miura and Takahashi 1988; Sonoda *et al.* 1992; Taylor and McPherson 1999; Taylor and McPherson 2003; Xiao and Tang 2007; Ditrich and Papáček 2010; Toubiana and Khila 2019; Matsushima *et al.* 2021), for the large-scale production of these insects. Several factors motivated our focus on protocol development. Firstly, previously proposed rearing methodologies lacked detailed and clear guidance on methods and materials. Secondly, the standardization of nymph age was not adequately addressed. Moreover, existing literature lacked adequate illustrations for egg, nymphal instars, and sex differentiation across life stages. Lastly, the remarkable potential of *M. pulchella* as a model organism for ecotoxicological and ethological studies, as well as its valuable role as a biocontrol agent in paddy rice fields, underscored the significance of our research. To augment the protocol, we incorporated essential components as follows: First, the development of a decision-making flowchart to optimize the rearing process of *M. pulchella*, aligning it with specific research objectives. Second, the implementation of a standardized method to accurately determine the ages of eggs and nymphs, ensuring meticulous experimental precision. Lastly, the inclusion of a visual representation illustrating all life stages and sexes of the insect, facilitating future investigations.

Furthermore, we provided an in-depth exploration of the life cycle and relevant life history characteristics of *M. pulchella*. Most of the measurements and assessments were performed under controlled laboratory settings, and we compared these results (when feasible) to those obtained from specimens collected in the field.

MATERIALS AND METHODS

Insect sampling

We used plastic strainers (21 cm diameter and 1.18 mm mesh eye) to capture adult *M. pulchella* insects in ditches, and stagnant water in paddy rice fields at the Centro Universitario Regional del Norte (CURDN) of the University of Tolima (Vereda Santo Domingo, Armero - Guayabal,

Colombia) in February 2022 (Fig. 1). Subsequently, the insects were carefully transferred to plastic containers (6 height x 8.5 width x 28 depth cm), half-filled with water from the collection site and transferred to the laboratory. *Microvelia pulchella* was identified using the taxonomic the key of Epler (Epler 2006).

Obtaining eggs from field-collected insects

Once in the laboratory, the collected adults of *M. pulchella* were housed in pairs (one male and one female) in petri dishes (9 cm diameter and 1.2 cm height) containing 15 mL of mineral water with a total of 200 pairs. In every Petri dish, a circle (7 cm diameter) of 180 g/m² white cardboard was placed over the water surface and kept still using adhesive tape at two opposite ends (Fig. 2a). The white colour of the cardboard facilitated the differentiation of adult insects; in addition, the eggs turn yellowish after two days and are easily recognized as well. The cardboard served as the resting site and oviposition substrate for *M. pulchella* adults. The environmental conditions in the lab were as follows, natural photoperiod (approximately 12:12 L:D), an average temperature of 25°C, and 68% RH. Insects were fed with frozen fruit flies, *Drosophila* sp. The flies were captured with traps placed in the field and used as bait ripe fruits such as banana, mango, lemon, and others; a single fly was daily placed in every Petri following the methods of Taylor and McPherson (Taylor and McPherson 2003) and Ditrich and Papáček (Ditrich and Papáček 2010). The fly carcasses were removed after 24 h before placing a fresh one.

Couples were kept in the aforementioned setup for 72 h, during which females laid eggs (mainly) on the cardboard. Subsequently, every insect couple was transferred to a new unit (similar to the previous one) to continue the process according to the required number of eggs and the number of field-collected couples available. This was necessary to avoid having nymphs hatching in the same units where the parents were housed; previous observations demonstrated that adult insects would cannibalize the nymphs when in crowded conditions (Andersen 1982, Miura and Takahashi 1988, Aristizábal-García 2017). Also, this method allows to standardize the age of the eggs and nymphs if the experiment requires it. Given that situation, the time window of egg collection can be reduced according to the availability

Figure 1. Methodology for collecting ***Microvelia pulchella*** adults in paddy rice fields.

of egg-laying females and their reproductive capacity (fecundity and fertility are discussed below).

Handling early nymphs

The egg-containing Petri dishes (from which the adult couples were removed) were inspected every 24 h to collect first-instar nymphs. These recently hatched insects were housed individually in 26 mL plastic containers (1.7 cm high and 4.4 cm in diameter) with 4 mL of mineral water and without cardboard (Fig 2b), this process was performed with the help of a plastic spoon. Water was renewed every six days, nymphs were fed daily with frozen *Drosophila* sp., and nymphs' exuviae and food debris were removed daily to avoid fungal proliferation. When the nymphs reached the adult stage, they were again placed in pairs in Petri dishes as described above for field-collected adults (Fig 2a). Only nymphs that hatched within a 24-hour time interval were used to standardize age and obtain data such as nymphal development, fecundity, fertility, and longevity. Such nymphs were separated into two cohorts; the first one (N=100) was used to determine the development time and obtain specimens for pictures. The second cohort (N = 64) was used for further measurements during the immature and adult stages. Both cohorts were from laboratory rearing but were divided into separate groups due to the need for specific insect manipulations, which were only applicable to the first cohort. Although it is possible to isolate the nymphs, manipulation of the early instars could cause damage to the insects. Consequently, the first cohort was used exclusively to collect data on nymph development, individualizing the nymphs. The second cohort was used to measure fecundity, fertility, and longevity (hence nymphs were kept in groups until adulthood to avoid damage).

Egg and nymph development

To determine the development time of the egg stage, we used a sample of 50 eggs (five groups of ten eggs each). The eggs were carefully removed from the cardboards previously placed in the petri dishes with the pairs. The cardboard was cut, previously locating the eggs and they were added in groups of ten to five Petri dishes. The eggs were inspected daily for over ten days. After hatching, nymphs were discarded. This measurement was independent of those made in the two cohorts mentioned above. The sole purpose of this sample of eggs was to determine the development time of *M. pulchella* eggs under controlled laboratory conditions.

Later, using the first cohort of 100 nymphs, each individual was individually housed in 26 mL plastic containers (as described above) immediately after eclosion. It is worth mention that although this method allowed us to keep records of every individual, manipulation of early instars is not recommended due to their fragility. If this manipu-

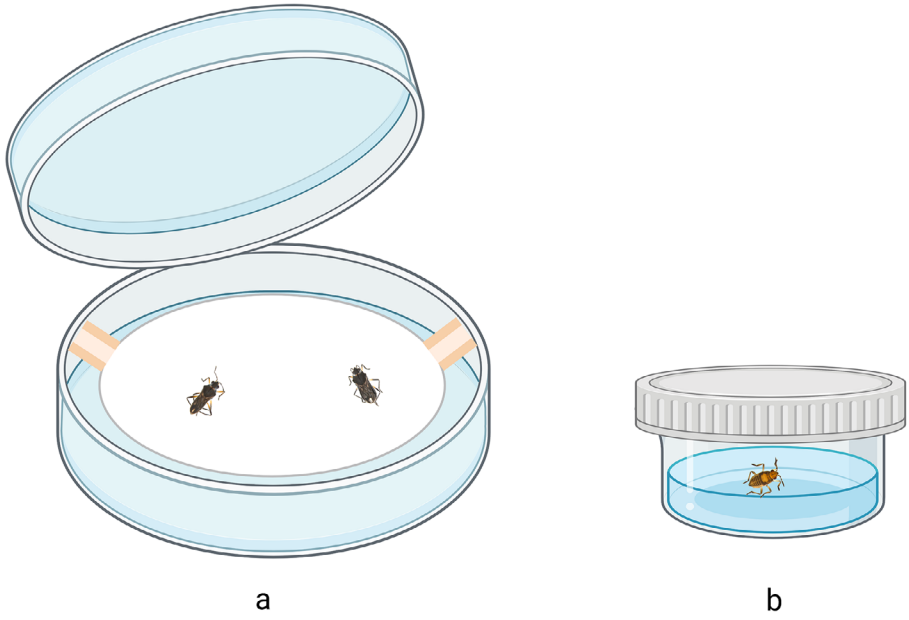

Figure 2. Containers used for rearing ***Microvelia pulchella***. a) Petri dish (9 cm diameter) for housing adult insects. b) Plastic container (26 mL) for rearing individual nymphs. Insects are not at scale.

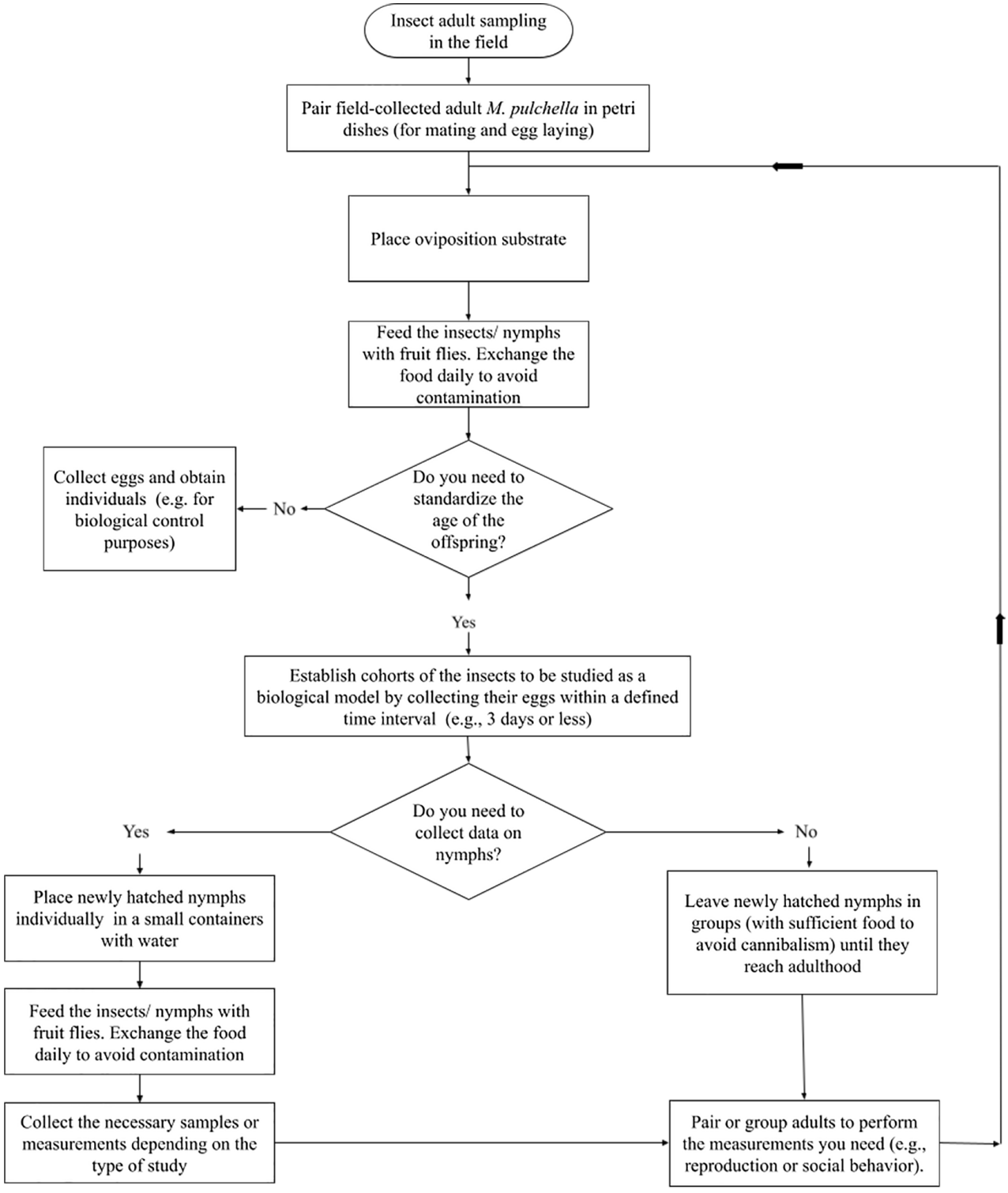

Figure 3. Flow chart of the ***Microvelia pulchella*** rearing protocol.

lation is necessary, then it is recommended to use plastic spoons, submerge them under the nymph, and catch water with the nymph to move the individuals without making direct contact with the individual. Each instar of the nymphs was identified by the presence of their exuvia in the container, as shown in Figure 4k. To create a reference collection for future studies and obtaining pictures, about ten insects were preserved in 70% ethanol after each moult. Additionally, each individual insect was easily isolated for measurements and observations.

Sex ratio, wing polymorphism, and body size in field and lab conditions

The second cohort of nymphs (N=64) was reared in lab conditions. However, at this time, nymphs were kept in groups (without the presence of the parents to avoid cannibalism) until they were eleven days old; at that age they were less prone suffer from mechanical damage during manipulation, in this way we ensured that the nymphs were healthy to evaluate fecundity, fertility and longevity. Afterward, all nymphs were individually housed in 26 mL plastic containers (Fig. 2b) and fed as described above. Once all individuals reached maturity, we recorded their sex and development type (i.e., winged or apterous). In addition, we collected samples (N=100 insects) of *M. pulchella* adults in distant areas of the paddy rice fields at the CURND. We recorded their sex and development type to confirm if the sex ratio and wing polymorphism was similar to that of the lab-reared insects.

To assess the body size of *M. pulchella* across several conditions (i.e., sex, type of wing, provenance), we took photos under a stereomicroscope (M205C, Leica Microsystems, Wetzlar) in a dorsal view of representative samples (lab-reared N = 63 and field-collected N = 90)"). We photographed adult insects of different sexes (i.e., male or female), provenance (i.e., lab-reared or field-collected), and development type (i.e., winged or apterous forms). The total length of every insect was measured from the tip of the rostrum to the end of the abdomen. Measurements were performed with the software ImageJ (Schneider *et al.* 2012).

Fecundity, fertility, and longevity

After *M. pulchella* nymphs of the second cohort reached adulthood (described above), we selected 20 males and 20 females and housed them in pairs (one female and one male) in 9 cm petri dishes using the setup described above (Fig. 2a). During the first week, the adult insects were kept separate, allowing a period of pre-oviposition. In this period, the females oviposited an average of 3.3 ± 2.09 ($\bar{x}$ ±SD). After the first week of adult life, we started recording weekly egg production (i.e., fecundity), and after every inspection, we transferred adult insects to a new experimental unit. By doing this, we kept track of the egg development process and avoided nymph cannibalism by the adult insects. Every egg-containing Petri dish was labelled and inspected for 10 days until no more eggs were hatched to calculate fertility ($\% = \#\, eggs\ hatched / \#\, eggs\ layed \times 100$). We repeated the same procedure for over twelve weeks (i.e., until no more eggs were laid) to track the fecundity and fertility throughout the adult stage. We used the same 20 pairs to determine the longevity of *M. pulchella* in lab conditions. The Petri dishes were inspected daily to record mortality. Carcasses of the adult insects were immediately removed, and the sex was recorded. All the steps described for collection and rearing protocol are illustrated in Fig 3.

Data analysis

All statistical analyses were performed in R 4.1.1 (Core Team c2019) using RStudio (RS Team c2020). Development time of eggs and nymphs, sex ratios and development type were analysed using descriptive statistics with the library Rmisc (Hope 2013). Insect size was analysed using linear models as data distribution was identified as Gaussian; in this case, insect provenance (i.e., lab or field), sex, and development type (i.e., winged or apterous) were included as fixed effects. Female fecundity (negative binomial distribution) and fertility (quasibinomial distribution) were analysed by means of generalized linear mixed effects models using the library lme4 (Bates *et al.* 2015), in both cases including the week as a fixed effect, and experimental unit as a random effect (due to repeated measures). Finally, insect lifespan was analysed with the Kaplan-Meier estimator using the library survival (Therneau c2016), including sex as fixed effect.

For all models, data distribution was identified using the library fitdistrplus (Delignette-Muller *et al.* 2019). Also, Type-II analysis of variance tables were used to assess the significance of terms using the 'Anova' function from the car library (Fox and Weisberg 2018). The library performance (Lüdecke *et al.* 2019) was used to inspect and plot the model diagnostics, and figures were made using the libraries sjPlot (Lüdecke c2016) and ggplot2 (Wickham 2016).

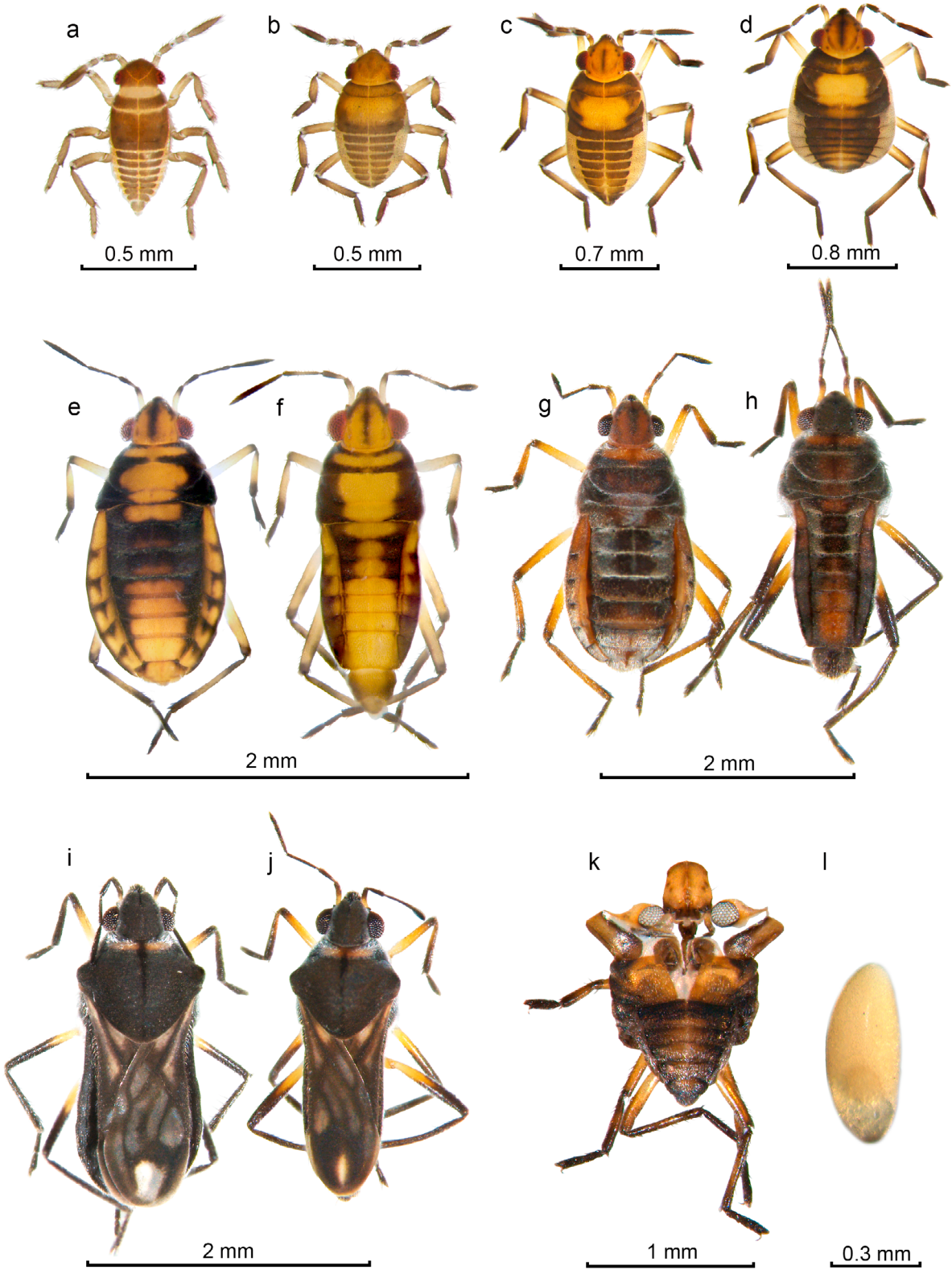

Figure 4. Life cycle stages and forms of ***Microvelia pulchella***. a) First instar nymph. b) Second instar nymph. c) Third instar nymph. d) Fourth instar nymph. e) Fifth instar female nymph. f) Fifth instar male nymph. g) Apterous adult female. h) Apterous adult male. i) Winged adult female. j) Winged adult male. k) Exuvia. l) Egg.

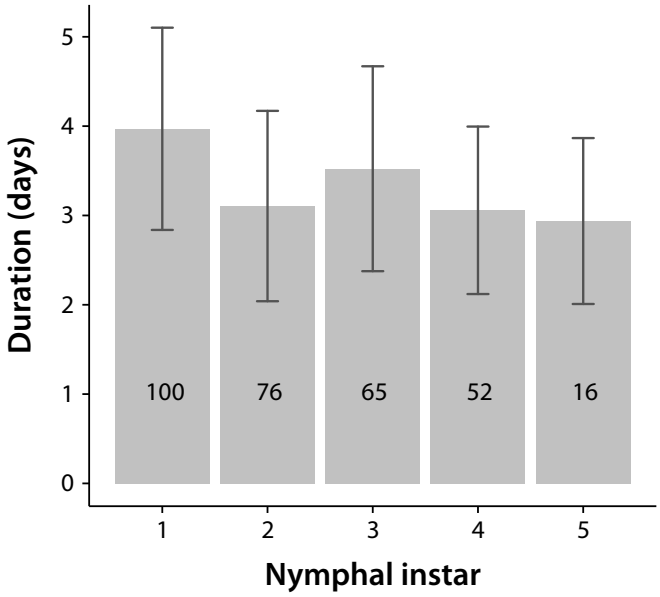

Figure 5. Average duration of every nymphal instar of ***Microvelia pulchella*** reared in lab conditions. Numbers in the grey bars represent the sample size (i.e., individuals) used for the calculation, and error bars represent the standard deviation.

RESULTS

Egg and nymph development

Eggs took 4.61 ± 0.49 days ($\bar{x}$ ± SE) to hatch under controlled lab conditions. Later, the nymphs reached adulthood within 16.00 ± 1.00 days ($\bar{x}$ ± SE) (considered after egg hatching) after going through five nymphal instars. Each one of these five instars lasted between 2.93 and 3.97 days on average (Fig. 5) and were easily discerned by the presence of the exuviae on the plastic container where the individual was housed (Fig. 4k).

Sex ratio, wing polymorphism, and body size in field and lab conditions

The sex ratio of *M. pulchella* was 0.70 in lab-reared and 1.59 in field-collected insects (♀ : ♂). This means that females were more abundant than males in field conditions when compared with the insects reared in the lab. Additionally, wing polymorphism patterns were markedly different depending on insect provenances. The percentage of apterous insects was 80.8% (females) and 75.7% (males) for lab-reared *M. pulchella*, and 6.5% (females) and 2.8% (males) for field-collected insects (Fig. 6).

On the other hand, body size (i.e., the length) of *M. pulchella* adults was significantly affected by the provenance (i.e., field or lab) and the sex in an interactive manner ($F = 21.71$, $P < 0.001$, Fig. 7a). Female size was consistent both in the field (2.35 ± 0.03 mm, $\bar{x}$ ± SE) and lab (2.38 ± 0.04 mm) conditions, while males exhibited a significant dif-

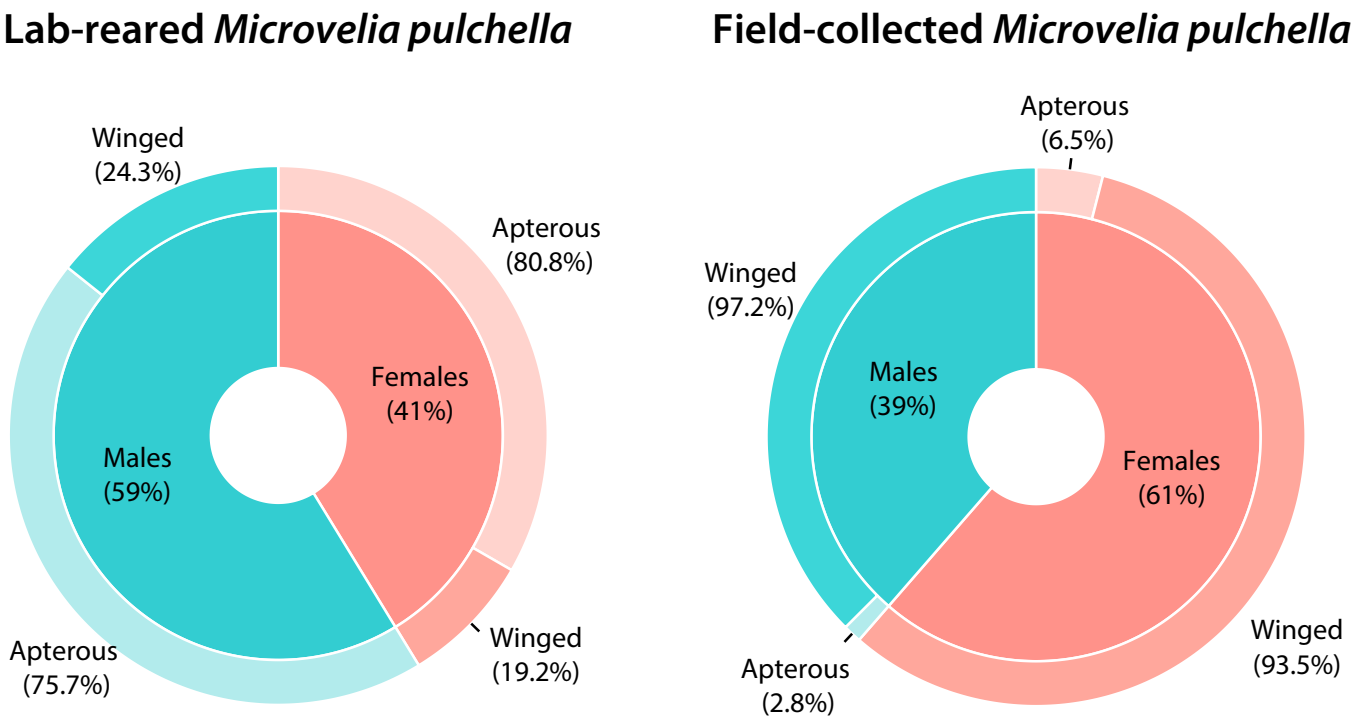

Figure 6. Sex ratio and wing polymorphism of ***Microvelia pulchella*** both in the lab and field conditions.

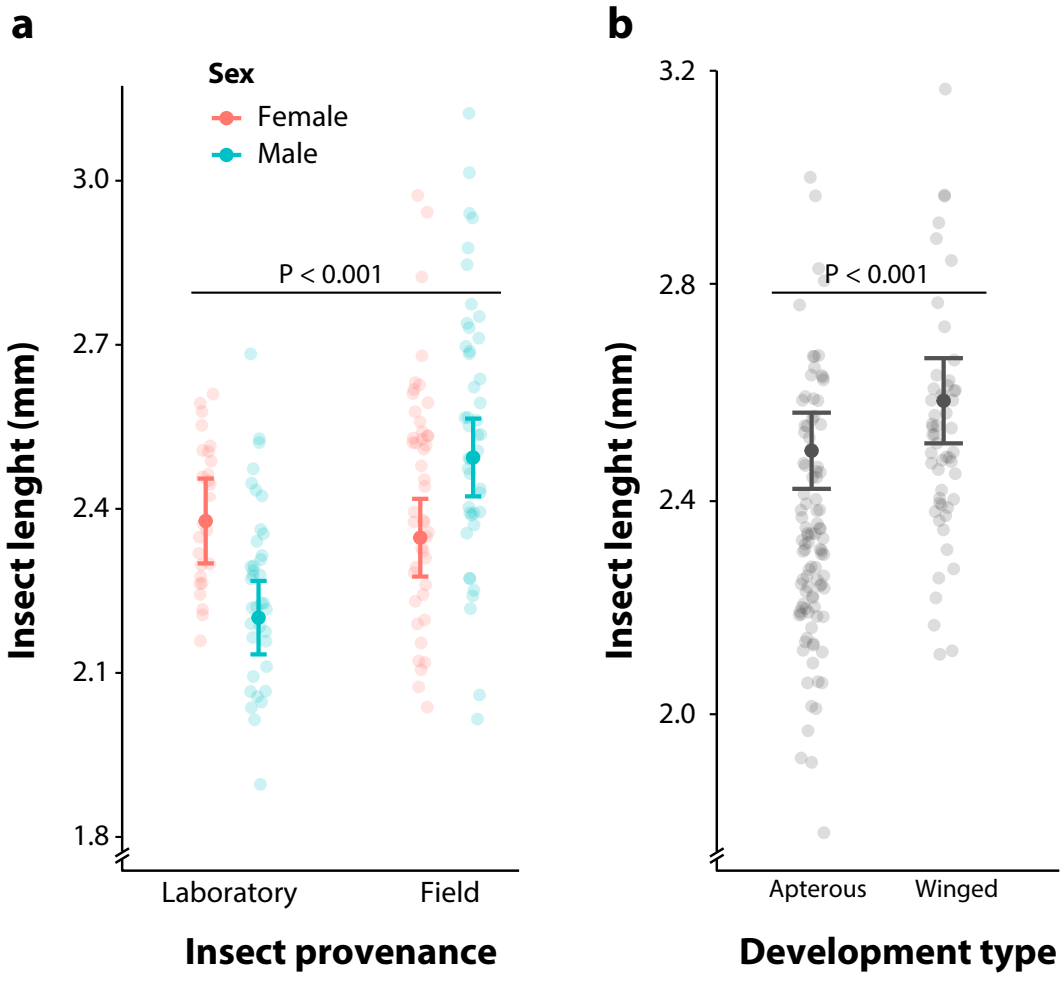

Figure 7. Body length in dorsal view of adult ***Microvelia pulchella*** (lab-reared N = 63 and field-collected N = 90). a) Comparison of body size between insect provenances. b) Comparison of body size between development type. The error bars depict the 95% confidence interval.

ference across conditions, being larger in the field (2.49 ± 0.04 mm, $\bar{x}$ ± SE) when compared with lab-reared insects (2.20 ± 0.03 mm). Moreover, the development type also affected insect size ($F = 23.98$, $P < 0.001$, Fig. 7b). In this case, apterous insects (2.27 ± 0.15 mm) were smaller than winged insects (2.43 ± 0.16 mm), regardless of sex and developmental conditions (i.e., field or lab).

Fecundity, fertility, and longevity

The fecundity of *M. pulchella* females significantly differed throughout their adult life ($\chi^2 = 258.72$, $P < 0.001$). *M. pulchella* started laying a significant number of eggs by the first week after being housed in couples (ca. second week of the adult stage), with an estimated 23.72 ± 0.38 (SE) eggs female^{-1} week^{-1}. Subsequently, in the second week, fecundity peaked at 37.51 ± 0.37 eggs female^{-1} week^{-1}, and it decreased gradually after the third week. By the sixth week and beyond, the fecundity was below twelve eggs female^{-1} week^{-1} (Fig. 8a). The total number of eggs laid by a *M. pulchella* female throughout their adult life was 211.77 ± 47.86.

In addition, the fertility of *M. pulchella* significantly decreased over time ($\chi^2 = 402.07$, $P < 0.001$, Fig. 8b). Around 79-95% of the eggs laid in the first five weeks hatched successfully. Between the sixth and tenth week, the fertility ranged from 56 to 34%, and afterwards, it was below 16%.

Regarding mortality and lifespan, it is worth stressing that nymphs of *M. pulchella* are very fragile during the first days after egg hatching. Due to the high risk of mechanical damage caused by the manipulation, we did not attempt to relocate nymphs of the second cohort to individual containers before they were eleven days old. During the first eleven days, nymph mortality was 26%; in this phase, with death mainly induced by fungi contamination due to food (i.e., fruit flies' carcasses) (Ramírez-Camejo *et al.* 2022). Furthermore, considering lifespan, males were longer living than females in lab conditions ($\chi^2 = 4.32$, $P = 0.037$).

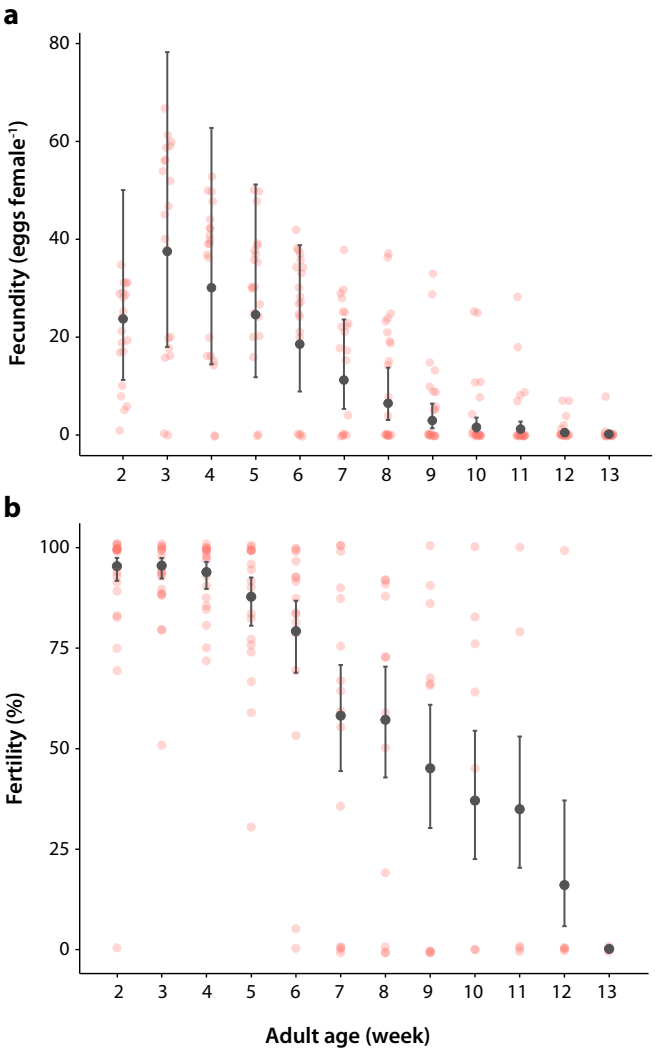

Figure 8. Reproductive parameters of *Microvelia pulchella* in lab conditions. a) Fecundity (i.e., eggs laid). b) Fertility (i.e., hatched eggs).

Based on elapsed time from egg hatching, females had a mean survival time of 74 ± 0.11 (SE) days, while males had a mean survival time of 91 ± 0.28 days (Fig. 9).

DISCUSSION

This study introduces a rearing protocol designed for the efficient mass production of *M. pulchella*, a species of broad-shouldered water strider. While previous studies have described methodologies for rearing *M. pulchella* (Nakasuji and Dyck 1984; Miura and Takahashi 1988; Sonoda *et al.* 1992; Taylor and McPherson 1999; Taylor and McPherson 2003; Xiao and Tang 2007; Ditrich and Papáček 2010; Toubiana and Khila 2019; Matsushima *et al.* 2021), these methodologies do not explicitly serve as comprehensive rearing protocols, resulting in certain knowledge gaps. Particularly, these methodologies lack guidance on tailoring methods to suit the intended use of the reared insects. Additionally, none of the previous studies provide sufficient photographic material for identifying nymphal instars and distinguishing between sexes (Taylor and McPherson 1999; Ditrich and Papáček 2010; Toubiana and Khila 2019). To address these shortcomings, our protocol incorporates a decision-making flowchart that optimizes the rearing process of *M. pulchella*, aligning it with specific research objectives. Furthermore, we provide a comprehensive plate of detailed photographs, facilitating the accurate morphological identification of each life stage and sex of the insect. Additionally, we outline a standardized procedure for determining the ages of eggs and nymphs.

Moreover, we compare the relevant life history traits of these insects under both laboratory and field conditions, ensuring a comprehensive understanding of their characteristics. We found that this insect species has a relatively short life cycle (compared to its congeners) (Taylor and McPherson 2003; Xiao and Tang 2007; Ditrich and Papáček 2010) and performs well in the lab regarding survival, fecundity, and fertility. Furthermore, we detected that controlled lab conditions affect the sex ratio, wing polymorphism patterns and size (only in males) of *M. pulchella* when compared with naturally occurring insects in paddy rice fields of the surrounding area.

In our implemented protocol, we suggest utilising two different-sized containers throughout the development of the insects depending on the space requirements in each stage (i.e., growth with a container of approximately 1.7 cm in height and 4.4 cm in diameter or reproduction with a container of approximately 9 cm in diameter and 1.2 cm in height). To avoid nymph mortality due to handling, we recommend using plastic spoons to transfer the nymphs to new containers when required. In addition, particularly at the earliest nymphal stages, it is required to keep the containers as clean as possible to avoid the proliferation of microorganisms due to exuviae and food (i.e., fly carcasses). Moreover, we detected that adult pairs had to be transferred to a new experimental unit after a maximum of six days to avoid nymph cannibalism. Considering all the daily tasks, maintaining a population of approximately 200 insects in lab conditions under our proposed protocol can be achieved in approximately two and a half hours. However, the task may take double this time when

experimental units are to be replaced. In general, we consider this to be a low-effort protocol, but tasks must be executed daily.

In this study, we recorded relatively high nymph mortality. We consider this to be mainly a consequence of contamination by microorganisms due to food, and not necessarily to naturally occurring mortality. Our implemented protocol used field-collected fruit flies, which may have traces of the fruit used to attract them. In order to avoid this issue, we recommend testing meat-based artificial diets (Saavedra *et al.* 1992). Additionally, previous studies have reported that density, drowning and incomplete ecdysis can induce mortality in the immature stages (Taylor and McPherson 1999, Ditrich and Papáček 2010).

Regarding immature development, *M. pulchella* has a relatively shorter development period than other laboratory-reared *Microvelia* species (Taylor and McPherson 2003; Xiao and Tang 2007; Ditrich and Papáček 2010). This rapid development is particularly noticeable for egg development time, as *M. pulchella* eggs only require 4.6 days at 25°C to fully develop, while other congeners require 6.5 – 9.8 days (Frick 1949, Taylor and McPherson 1999, 2003, Xiao and Tang 2007). Similarly, the nymphal development time of *M. pulchella* (i.e., 16 days) was among the shortest for the genus *Microvelia* when compared with its congeners (13.9 to 24.22 days, in temperatures between 20 and 26°C) (Frick 1949, Muraji and Nakasuji 1988, Taylor and McPherson 1999, 2003). It is well known that environmental factors significantly affect insect development (i.e., temperature, nutritional resources, photoperiod, and crowding) (Spence *et al.* 1980, Moreira 2015, Gutiérrez 2020). As a result, some conditions may be adjusted to control to some extent biological traits of the insects. For instance, microveliids subjected to 32°C environments had a nymphal development period as short as nine days (Muraji and Nakasuji 1988), and *M. douglasi* nymphs had a faster development (ca. fifteen days) when fed with a diversified diet (Sonoda *et al.* 1992). Considering the abovementioned, further experiments with *M. pulchella* would allow adjusting the rearing conditions (e.g., temperature and food) for optimal nymphal development.

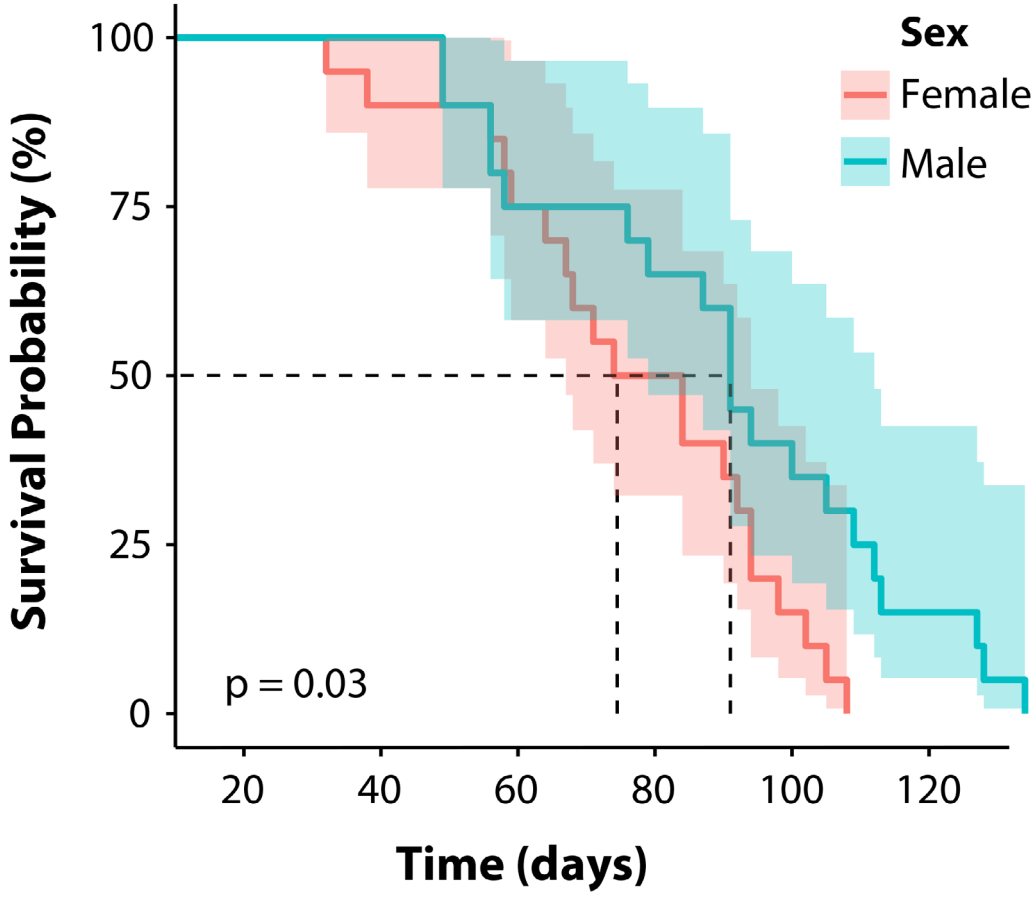

Figure 9. Lifespan of *Microvelia pulchella* in lab conditions. The lifespan of both male and female insects did not differ.

When insects reached maturity, we detected that lab-reared males were smaller than their field-collected counterparts. It is likely that food availability played an important role in this phenomenon, as we only provided one prey (i.e., fruit-fly) per day, and that portion may have been insufficient for males. However, this effect was not the same in females since their sizes both in the field and in the laboratory did not differ significantly. More studies are needed to identify the nutritional requirements of each sex in this species since, in general, nutritional interests depend on disparate life strategies (the female invests in reproduction and the male invests in secondary sexual traits) (Morehouse *et al.* 2010; Gutiérrez *et al.* 2020). Insects of the species *M. pulchella* are opportunistic predators and scavengers (Moreira 2015) and in field conditions may have access to a higher quantity and a more diversified diet. Therefore, we further stress the need to experiment with diet composition and abundance. Moreover, insects reared in controlled conditions had a higher proportion of males than females, and individuals of both sexes were predominantly apterous. Conversely, field populations appear to be female dominated, and most insects exhibit complete wing development. The cause of the inverted sex ratio in controlled conditions cannot be deduced from the collected data and must be elucidated through future studies. One could argue that the presence of some microorganisms in the field may favour the female sex (Kageyama *et al.* 1998). However, it is also very likely that there is a bias in the estimation of sexes in natural populations due to secondary factors such as differential mortality of the sexes in the environment (which might affect males more than females) and differential distribution of males and females over time and/or across habitats (Tabadkani *et al.* 2013).On the other hand, obtaining a high proportion of apterous insects under controlled conditions can be considered a good indicator of favourable conditions for these semi-aquatic insects, as it was previously pointed out that veliids usually develop wings to leave unsuitable habitats (Harrison 1980, Muraji and Nakasuji 1988, Jones and Coleman 1991, Ditrich and Papáček 2010). Also, apterous *Microvelia* females have been reported to have increased fecundity and fertility (Muraji *et al.* 1989, Matsushima and Yokoi 2022), which is a desirable trait for the mass production of this insect species. However, it is important to note that mass rearing for subsequent release of insects should be done with caution, based on previous studies, to avoid imbalances in the ecosystem.

Regarding the reproductive traits, *M. pulchella* had a similar fecundity (ca. 212 eggs) and oviposition period (56 days) to that reported for other species within the genus *Microvelia* (Muraji and Nakasuji 1988, Taylor and McPherson 1999). However, the species *M. horvathi* can lay up to 450 eggs during their reproductive period (Muraji and Nakasuji 1988). It would be interesting to investigate whether manipulating the rearing conditions or mating frequency can further increase the fecundity of *M. pulchella* females. In this study, we allowed a seven-day pre-oviposition period before coupling adult insects following the previous studies (Nakasuji and Dyck 1984, Muraji and Nakasuji 1988, Taylor and McPherson 1999). However, due to wing polymorphism, it can be difficult to differentiate adult females from mature nymphs at first glance. Therefore, relying on detecting the exuviae and the consistent number of nymphal instars is a good strategy to identify when the insects reach adulthood.

Furthermore, the temporal pattern of the fertility exhibited by the eggs laid by *M. pulchella* may suggest that females should only be used for obtaining eggs during the first five weeks of their egg-laying period. Nevertheless, considering that couples were monogamous, it is possible that sperm viability of male *M. pulchella* decreased rapidly with age, as occurs with other insect species (Stürup *et al.* 2013, Tasnin *et al.* 2021). Therefore, a single male would not be sufficient to continue to fertilize the eggs during the entire female reproductive period. A strategy to overcome this issue would be to replace the males after four weeks for younger ones. However, this topic deserves further investigation as the fertility directly determines the success of the mass-production efforts. On the other hand, concerning the longevity exhibited by *M. pulchella* individuals, it was consistent with previous studies that suggest that individuals of *Microvelia* normally live approximately three months (Muraji and Nakasuji 1988, Taylor and McPherson 1999). This relatively short life cycle is also a desirable trait in a model organism (Ankeny and Leonelli 2011), mainly for ecotoxicological (Rico *et al.* 2011; Hayasaka *et al.* 2015; Liu *et al.* 2016) and ethological studies (Ohba *et al.* 2011; Ditrich and Boukal 2016; Toubiana *et al.* 2021; Matsushima and Yokoi 2022).

Overall, our study demonstrates that the proposed protocol for rearing *M. pulchella* in laboratory conditions is both simple and cost-effective. With a wide distribution and easy-to-collect individuals, *M. pulchella* presents it-

self as a viable model organism for bioassays and experiments Unlike other aquatic and semiaquatic invertebrates, which require strict diets and special materials for rearing, *M. pulchella* is a generalist predator and does not depend on a single type of prey. Our study also shows that informative traits, such as reproductive performance, can be measured in a non-invasive way. While previous studies have revealed some of these advantages, our results provide new insights particularly with regards to the handling of eggs and newly hatched nymphs. Additionally, we introduce practical tools, such as a flowchart and images of the insect, to facilitate its rearing process, tailored to specific research goals.

AUTHORS' PARTICIPATION

AAB conceptualization, data collection, writing-original draft, and writing-review and editing. GB conceptualization and data collection. TB conceptualization, supervision, funding acquisition, project administration, and writing-review and editing. YG conceptualization, supervision, data analysis, writing-original draft, and writing-review and editing.

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