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RESEARCH ARTICLE

BugBook: Basic information and good practices on how to maintain stock populations for *Tenebrio molitor* and *Hermetia illucens* for research

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Abstract

The information on the production and reproduction of *Tenebrio molitor* and *Hermetia illucens* is essential for researchers pursuing a career in insect farming for food, feed and non-food applications. Rather than requiring researchers to sift through numerous studies to develop effective rearing protocols to maintain a stock population for experimental purposes, the authors summarized insights from peer-reviewed research, while expanding it with their collective experience in rearing these species in both laboratory and pilot-scale settings. A similar approach was applied to both species, beginning with a detailed overview of their life cycle, which is used as a framework for various maintenance procedures. Followed by a description of the environmental conditions in which each species should be held and the minimal infrastructure needed to rear them. Feed recommendations are provided, along with good practices for each stage of the lifecycle, including egg production, instar rearing, and adult handling. The final section addresses potential risks and hazards associated with insect rearing. This includes concerns about the allergenicity of particles produced during rearing, as well as the possibility of contamination, disease outbreaks, or pest infestations. The risks associated with maintaining different insect strains, including cross-contamination and the potential for genetic drift or inbreeding depression, are also discussed.

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Keywords

black soldier fly – breeding – BSF rearing – mealworm

1 Introduction

Research on insect farming for feed, food and non-food has been increasing over the last decades as was reviewed for *Hermetia illucens* by Athanassiou *et al.* (2025) and for *Tenebrio molitor* by Ribeiro *et al.* (2018), Gkinali *et al.* (2022) and Kotsou *et al.* (2024). For a researcher pursuing this topic, maintaining a steady stock population is essential as it serves as the primary source of insects for experimental purposes. In this article, information is provided on how to maintain a basic experimental and healthy population of *Tenebrio molitor* and *Hermetia illucens* and the accompanying risks and hazards. The information provided below is a mixture of published results and experience from the authors who maintained insect populations.

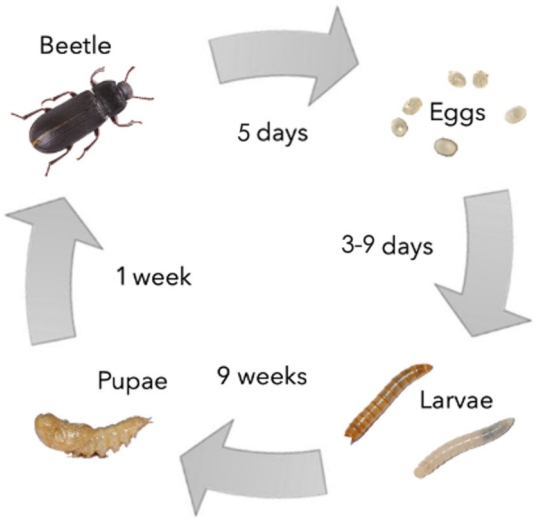

FIGURE 1 Life cycle of *Tenebrio molitor*. The approximate time they are in each stage is indicated by the arrow next to their picture. The presented time is at 27 °C but can vary depending on environmental conditions and feed used.

2 The yellow mealworm (*Tenebrio molitor*)

Life cycle of the mealworm

Tenebrio molitor or the yellow mealworm is a black beetle belonging to the Tenebrionidae family. The primary objective of mealworm production is the harvesting of the larvae, which are commercially known as mealworms. The life cycle of the mealworm comprises four distinct metamorphic stages: egg, larva, pupa, and adult (imago). Cotton (1927) provided one of the earliest comprehensive overviews of this life cycle, noting that environmental factors (temperature, humidity, food and water) can significantly influence the duration of each stage.

The egg stage is characterized by the production of small bean-shaped, sticky, white eggs (about 1.5 mm in length). This stage is the shortest in the life cycle. At 25 °C, Siemianowska *et al.* (2013) described eggs hatch after 3-9 days, coinciding with the findings of other authors who have indicated similar durations within the 25-35 °C temperature range, as reviewed in Ribeiro *et al.* (2018). Nevertheless, this duration may be extended up to fourfold at lower temperatures (Kim *et al.*, 2015).

The larval stage, however, can extend up to 3-4 months depending on the rearing conditions and feed availability (Park *et al.*, 2014). Currently, under favourable environmental and nutritional conditions, the larval growth period has been reduced to 9 weeks (Pascual *et al.*, 2024a). Larvae experience between 9 and 23 instars (Ribeiro *et al.*, 2018), increasing in size from a few mm to around 3 cm at the 20th instar (Park *et al.*, 2014). Following the moulting process, the larvae are initially soft and white in colour, but subsequently harden rapidly.

After completing the larval stage, the mealworm forms pupa in the final moult, which typically lasts from 5 to 7 days (Morales-Ramos *et al.*, 2010; Siemianowska *et al.*, 2013). Although less common, maximum longer durations up to 18-20 days were reviewed in Ribeiro *et al.* (2018). Mealworms produce an immobile white pupae with a soft exoskeleton, without mouth nor anus. During the pupa stage, the insect undergoes internal and external transformation into an adult.

The adult's lifespan according to Cotton (1927) is 38 days. This may vary from 17 to 173 days, as reviewed by Ribeiro *et al.* (2018). Upon emergence from the pupa, the beetles are initially pale in colour and possess soft exoskeletons. As they mature, the beetles darken and harden, after which they reproduce and complete their life cycle. The adult beetle has an approximate length of 2.5 cm. The entire life cycle is visualized in Figure 1.

Infrastructure and resources

Rearing room requirements

Maintaining a stock population of mealworms can be done in any room that can store crates, as crates (of various sizes) are the main recipients in which all stages of mealworms are housed. Stackable crates can be used or crates can be arranged in racks or shelves. Both systems however have to provide sufficient air flow between the crates. On a pilot scale, crates of e.g. 40 × 60 cm (or smaller) can be used. Larger crates are discouraged due to inconvenience when handling manually. Insects are ectothermic, thus mealworms rely on external conditions to regulate their body temperature, making at least heating above room temperature essential. Small deviations in average temperature, as minor as 2-3 °C, can significantly affect development and growth (Ortiz *et al.*, 2016). However, when the mealworms are mainly there to generate new offspring for experimental purposes, there is a trade-off between maximizing the output of a room and the costs associated with strictly controlling environmental factors. Temperature, relative humidity (RH) of the ambient air, as well as ventilation rate and air refreshment rate, are all known to have an effect on mealworms (Veldkamp *et al.*, 2021) and are detailed in the following paragraphs.

Bjørge *et al.* (2018) observed that *T. molitor* larvae exhibit a net positive growth between 15 and 39 °C, with a maximum at 31 °C. However, ambient air is best kept below 31 °C, as Deruytter *et al.* (2022) observed a net temperature increase inside a mealworm crate related to the larvae biomass. When heating ambient air to 31 °C, the optimum would be overshot inside the crate with approximately 2 °C per kg of larvae biomass in the crate, leading to production losses rather than gains. As for oviposition ShiCai *et al.* (2012) found that the optimal temperature range was between 26 and 29 °C and an optimum of 27.8 °C was determined for larvae growth and development. Eberle *et al.* (2022) observed optimal conditions that yield the highest survival rate, fastest growth, and shortest developmental time at 25 °C and 30 °C under constant darkness. However, Coudron *et al.* (2022) compared mealworm production between a greenhouse setting and a room with strict climate control. Both locations had a similar average temperature (27 °C). The greenhouse experienced a 17 °C temperature difference between the coldest and warmest moment, compared to the 2 °C difference under controlled conditions. Nonetheless, only a 7.5% productivity loss was observed in the greenhouse setting compared to the controlled setting. This is not only the case for larvae growth, also egg hatching tolerates a range of ambient temperatures (Deruytter *et al.*, 2023), suggesting that some flexibility around an above room temperature average can be allowed.

According to different studies, optimal RH for mealworm oviposition lays between 60 and 75% (Manojlovic, 1987; Punzo and Mutchmor, 1980). In the study by Johnsen *et al.* (2021), various RH levels were tested to assess their impact on mealworm larvae. While RH had no significant effect on survival rates, the larval mass and length increased with higher humidity. Larvae reared at 84% RH were 1.96 times heavier and 1.31 times longer than those raised at 43%. However, maintaining such high humidity can cause issues as moulds and mites (e.g. the flour mite, *Acarus siro*) proliferate under these conditions, and is therefore not recommended. For yellow mealworm, therefore RH is often set at 60% (Ortiz *et al.*, 2016; Ribeiro *et al.*, 2018; Veldkamp *et al.*, 2021).

It is recommended to design the rearing room to enable efficient climate control. Temperature is allowed to fluctuate several degrees but is better to be on average at least 25 °C, although colder average temperatures are not lethal, higher temperatures will shorten the mealworm's lifecycle. An option to (de)humidify air to around 60% RH is also recommended. Additionally, ventilation will help mixing the air, countering undesirable microclimates while removing metabolic CO_2 from the mealworms, ensuring a safe working environment. Climate chambers should avoid high ceilings, as these increase energy consumption and production costs due to heat loss.

Feed requirements

To optimise insect health and production, nutrients should be applied in the appropriate form and quantity tailored to each specific stage and physiological phase of their life cycle, excluding the pupal stage. The adult beetles do not exhibit a voracious appetite, however, an adequate food supply during this phase can influence the fertility and life expectancy of the beetles (Rho and Lee, 2016). During the larval growth phase most of the feed is utilized. In this section we will address the necessities regarding dry and wet feed for *T. molitor*, without delving into the nutritional requirements in this section as a separate BugBook article (Oonincx *et al.*, 2025) addresses this topic more extensively.

Dry feed

Dry feed represents the primary source of macronutrients in the diet of mealworms. To illustrate, in a diet comprising 48% wheat bran and 52% carrot, 88% of

the digestible energy and 90% of the digestible protein is derived from wheat bran (calculated from Fasce *et al.* (2022) and Pascual *et al.* (2024b)). When using agar gel as a wet feed source, the allocation is getting even more skewed.

As their name indicates, mealworms have a particular affinity for grain-based "meal" products. Kröncke and Benning (2022), observed that the most suitable substrates for mealworm rearing are often cereal-based products (comprised of fine or coarse granular particles or flakes), varying in size and texture. These include wheat bran and flour, maize hulls, oat bran and flakes, rice flour, lupine flour, and potato flakes. It is also important to consider that the growth rate of the larvae seems to be inversely proportional to the particle size of the feed, with the greatest growth occurring when particle size is below 2 mm (Naser El Deen *et al.*, 2022). Morales-Ramos *et al.* (2011) indicated mealworms are capable of self-selecting between wheat bran and dry potato flakes in a two-diet mixture, approaching the optimal ratio for population growth. Moreover, some data indicates that they also regulate this selection based on the nutrients provided. Kröncke and Benning (2022) observed that larvae require carbohydrates and protein to a greater extent than fats and minerals. In the case of the mealworm, a ratio of approximately 1:1 for protein and carbohydrates was selected over the initial 15 days of the experiment (days 0-15). However, a preference for carbohydrate-like feed was exhibited over the subsequent 15 days (Rho and Lee, 2022). As for the adult stage, when provided with the option of combining two nutritionally imbalanced but complementary foods, the beetles demonstrated an active regulation of their intake of protein and carbohydrates, achieving a ratio of 1:1 (Rho and Lee, 2014).

Mealworms are best reared in a dry, loose, granular, coarse-textured bedding or substrate that provides a source of fibrous feed, such as wheat bran. The grainy texture allows them to burrow and helps with moisture regulation. This facilitates larval mobility and easy access to feed (Pascual *et al.*, 2024a). This structure also facilitates the larvae's access to the bran, which is their preferred feeding substrate (Murray, 1960) and also a perfect simple feeding substrate for basic population maintenance.

Wet feed

Murray (1968) observed that the difference between mealworms given access to water and those deprived of it becomes progressively more clear with the reduction of humidity levels. While mealworms can technically survive without a direct water source if the air is humid enough (Machin, 1975), this is discouraged in practice as is discussed in the section on Rearing room requirements. An RH within 40 to 85% does not typically exert a considerable influence on larval survival (Johnsen *et al.*, 2021), access to water can significantly impact on larval mass obtained during the initial weeks of life (Oonincx *et al.*, 2015b; Ribeiro *et al.*, 2018; Urs and Hopkins, 1973). Therefore, providing a manageable water source via their feed (e.g. fresh vegetables such as carrots, agar gel, apples, …) is a better alternative to relying solely on atmospheric water. Furthermore, fresh products are often a source of micronutrients (trace elements and vitamins) that can help meet the nutritional requirements of insects.

Given the mobility constraints of mealworm larvae, it is crucial to develop a strategy that ensures all larvae have continuous access to water throughout their development. When dealing with smaller larvae, it is necessary to use smaller and more evenly distributed pieces, with more frequent replacement. Deruytter *et al.* (2021a) recommend the placement of wet feed within 5 cm for the larvae to ensure that all larvae sizes grow equally. Finally, wet feed is prone to decay. It should be given proportionally to the larval biomass in the crate so that it is readily consumed. Regularly monitor the amount of wet feed given and adjust the feed based on the number and size of larvae.

Good practices per stage

Production of eggs

In order to optimize the reproductive output of *T. molitor*, specific strategies and best practices need to be applied. The goal of these practices is to increase the efficiency of *T. molitor* rearing and thereby reduce operational time. In recent years significant efforts have been directed towards maximizing productivity, particularly in terms of offspring production. Research indicates that female *T. molitor* beetles become sexually receptive approximately five days after emergence. At this point, they are capable of mating with various males multiple times, as demonstrated by studies from Gerber and Sabourin (1984) and Worden and Parker (2001). Interestingly, female weight does not appear to have a significant impact on fecundity in *T. molitor* (Morales-Ramos *et al.*, 2012), suggesting that other factors, such as age, environmental conditions, and mating frequency, play more pivotal roles in determining reproductive success.

One of the factors that affects reproductive success is the age of the adult beetles. Various studies, including those by Adamaki-Sotiraki *et al.* (2023), Berggreen *et al.*

(2018), Frooninckx *et al.* (2022) and Morales-Ramos *et al.* (2012), highlighted that beetles tend to lay fewer eggs as they age, similarly to other insect species. Morales-Ramos *et al.* (2012) reported that reproduction typically reaches a peak during the second week after emergence and remains high until the third week. After this period, a decline in reproduction is evident, they recommend replacing adults every 58-74 days after eclosion as they have reached 80-90% of their oviposition potential by then. Similarly, Berggreen *et al.* (2018) described that the peak reproductive age occurs between 13 to 29 days, with a sharp decline in fecundity between 30 and 39 days of age.

Apart from beetle age, oviposition time is crucial as well. Variation in growth between individuals is inherent to biology. However, minimizing the oviposition period can at least reduce this source of variation for future experiments. Deruytter *et al.* (2019), suggested limiting it to 4 days to maintain uniformity in larval development. A 7-day oviposition time only slightly increased the variability of the larvae. In combination with the ease of mealworm maintenance planning as it fits in a week, a 7-day oviposition period is recommended for general population maintenance.

Adult density within a rearing facility is a variable that can be manipulated relatively easily. However, the relationship between density and offspring production is complex. High adult densities do not necessarily yield maximum reproductive output per unit of rearing area (Halliday *et al.*, 2015). In fact, studies indicate that overcrowding among adults often results in reduced (per female) reproductive rates, a phenomenon seen across other species in the Tenebrionidae family. This could be attributed to disturbance but also to beetles cannibalising conspecific eggs as discussed in the next paragraph. Therefore, there is a trade-of between maximising the per female output and the per crate progeny output. Berggreen *et al.* (2018) tested adult densities up to 0.84 beetles per cm^2, with the highest density resulting in the highest offspring count per crate. While Deruytter *et al.* (2019) tested up to 2.8 beetles/cm^2 with still increasing offspring production per crate. However, both authors noted that the highest per female offspring production occurred at the lower densities. Although high densities in a controlled environment seem to negatively impact the per female egg production, observations in nature reveal that *T. molitor* beetles tend to form dense aggregations, suggesting that the reduction in reproductive output in captivity may be more related to environmental stressors like limited food availability and suboptimal temperatures rather than density *per se*. This theory

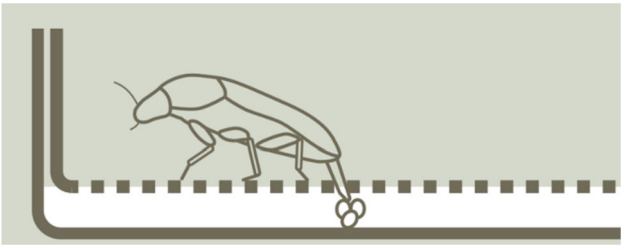

FIGURE 2 Schematic representation of a beetle pushing its ovipositor through the sieve, leaving its eggs out of reach from other beetles.

is supported by studies conducted by Berggreen *et al.* (2018) and Halliday *et al.* (2015), which emphasize the importance of environmental conditions in regulating reproductive success.

Cannibalism of eggs by adults is a behaviour observed in many Tenebrionidae species, including *T. molitor*. As reported by Halliday *et al.* (2015) and Longstaff (1995), egg cannibalism is believed to regulate offspring production, preventing overcrowding and resource depletion in natural populations. However, in rearing facilities, cannibalism reduces the overall number of viable eggs and, subsequently, the number of larvae produced. To mitigate this issue, researchers such as Berggreen *et al.* (2018) have proposed separating adult beetles from eggs using a sieve (2 × 2 mm) as illustrated in Figure 2. This method has been shown to significantly increase the number of eggs produced and prevent cannibalism, as confirmed by Deruytter *et al.* (2019) and Frooninckx *et al.* (2022). Such oviposition system is now widely suggested for mealworm reproduction (Coudron *et al.*, 2019; Spranghers *et al.*, 2021).

Combining the aforementioned insights, we propose some good practices concerning egg production. In this example a standard 60 × 40 cm crate is used as a reference. Initially, around 2000 sexually mature beetles (the equivalent of around 250 g) are placed on top of an egg sieve (mesh 2 × 2 mm) in a crate. 2000 g of wheat bran should be provided ensuring there is both feed above and below the sieve. The dry feed will serve as bedding material for the eggs, feeding substrate for the adults and for the young mealworms during their first few weeks. It should be ensured that the sieve is embedded in the substrate without any air pockets, allowing the adults to oviposit their eggs. During the oviposition period, adults are provided with *ad libitum* wet feed (i.e. vegetables or a gelling agent). After 7 days of oviposition, the adults should be removed and the eggs are left to eclose and the neonate mealworms to feed for approximately two weeks. The aforementioned procedures are presented schematically in Figure 3. By replacing the

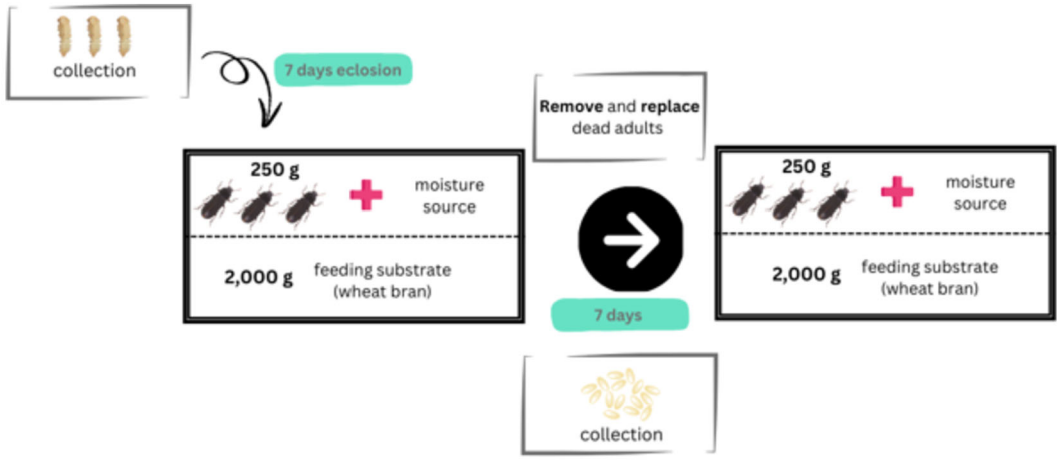

FIGURE 3 Schematic presentation of the rearing procedures of *Tenebrio molitor* adults for egg production.

feeding substrate with for example wheat flour (particle size < 0.5 mm), it is possible to sieve off the flour using a 0.5 mm sieve and retrieve relatively pure eggs, something that is impossible with a coarser oviposition substrate.

Rearing instars

After the initial 2 week nursing period post oviposition, in which the eggs and larvae are left undisturbed, it is recommended to provide the mealworms with wet feed at regular intervals, as described in the feed requirements section above. One critical factor to manage at this stage is the density of mealworms, as it directly influences their growth. The expected number of offspring from 2000 beetles (approximately 250 g) can vary significantly as was discussed in the previous section. Assuming an oviposition period of 7 days in a 60 × 40 cm container, with a 1:1 female-to-male ratio and the use of an egg sieve (to minimize cannibalism), the progeny count can reach up to 84 000 mealworms. In the absence of an egg sieve, this number may be as low as 14 000 mealworms (Deruytter *et al.*, 2019).

A general guideline is to rear larvae in two steps, first at the density at which they were initially oviposited and leave them at this density for the first 4 to 5 weeks. Followed by a redistribution to a desired density for a grow-out phase. When redistributing, it is essential to estimate the size of the mealworm population. Deruytter *et al.* (2024a,b) describe a method for this estimation. First, sieve the frass using a 0.5 mm mesh, then weigh the larvae and remaining feed. Mix the contents gently but thoroughly, and take three samples containing at least 100 mealworms. Weigh the samples and count the mealworms in each one. The average number of mealworms per gram provides an accurate estimate of the total number available. After redistribution to the desired density, add sufficient dry feed to support the larvae in reaching the desired average weight or completing their life cycle.

The appropriate larval density for rearing varies depending on the intended use of the larvae, such as for experimental purposes (as detailed in Deruytter *et al.*, 2025) or for maintaining stock for future breeding. While mealworm harvests can reach up to 4.2 kg per 60 × 40 cm crate with 64 000 mealworms (Deruytter *et al.*, 2022), individual performance tends to decline at these densities. This decline is linked to factors that negatively impact mealworm welfare and growth rates, including rapid feed depletion, frass accumulation and temperature increase to lethal thresholds. The study recommended to rear mealworm at a density of 1 mealworm per cm^3 of feed (in the case of wheat bran). However, this was driven by economical considerations as best growth was observed at lower densities of only 1 mealworm per 2 cm^3. If the objective is to produce new adults, it is recommended to maintain a relatively low density, such as 5000 mealworms per 60 × 40 cm crate. Although this density may not be the most economically efficient, unpublished data indicates that higher densities delay pupation, and mealworms that do pupate may succumb to cannibalism.

During the grow-out phase, sieves with mesh sizes of 1.0 mm, 2.0 mm and 3.0 mm can be useful for separating older or larger mealworms from their substrate. However, removing frass or substrate from the crate is not strictly necessary. At a density of 5000 larvae per crate (60 × 40 cm) they can grow until pupation without interference. It may take 4 to 5 weeks before the first pupae will form, however it can take up to 4 weeks more for 95% of the mealworm to pupate.

FIGURE 4 A bar sieve or fish grader, used to sieve pupae from the larvae.

FIGURE 5 Separation procedure of live and dead adults of the insect species *Tenebrio molitor*.

New adults

Due to the cannibalistic behaviour of mealworms, newly formed pupae should be isolated from larvae to avoid damage caused by larval movement or chewing. It is recommended to, at least, collect pupae every week as otherwise new beetles will appear. Furthermore, due to the variability in pupation rate, pupae can be collected from the same crate for several weeks. To successfully and efficiently separate larvae and pupae, a sieve with a non-square mesh can be used (Figure 4). For example, a fish grader or a wire sieve with a 3 to 3.5 mm aperture can be used for manual sieving or a mechanical sieve with a 3.5 × 35 mm rectangular mesh. The exact dimensions can be specific to one's specific population characteristics. The necessary mesh size can easily be determined by measuring the length and width of fully grown mealworm larvae and the width of the head of the pupae. It should be longer than the longest mealworm and narrower than the head of most pupae.

The collected pupae are delicate and should be kept in a single layer. They can be kept in crates in the same environmental conditions as the other life stages. The pupae can be left undisturbed for at least 7 days for the pupae to eclose and the beetles to mature. These fresh adults can thereafter be used to replenish the adult population after the dead beetles are removed.

The procedure of replacing dead with live adults in an insect population is essential in order to keep it healthy and makes sure that egg production remains consistent over time. The behaviour involving the removal of dead individuals from the nest to prevent disease transmission, also called necrophoresis, is mainly performed by social insects (i.e. ants, bees, wasps, and termites) (López-Riquelme and Fanjul-Moles, 2013). The case of social insects aligns with the case of mass-rearing of insects for food and feed as dense groups of closely related individuals with frequent physical contact create ideal conditions for the spread and occurrence of infectious diseases. Dead individuals within a population pose a significant epidemiological risk (Cremer *et al.*, 2007). Interestingly, a recent study applied this concept in mealworm production, using the removal of *T. molitor* adults to stabilize population numbers, which in turn helped maintain consistent egg production (Adamaki-Sotiraki *et al.*, 2024).

Based on the above, adults from each crate should be carefully separated from their egg-sieves after oviposition to combine them into a single crate. Following this, each egg-sieve should be cleaned thoroughly to remove any residual egg material or other debris. To remove dead adults from the population, the beetles (live and dead) can be placed on a sieve mesh (Figure 5) or an egg carton. Live beetles will cling onto anything within their reach and by gently pouring off everything that is still loose, dead individuals will be disposed. This procedure might require a few repetitions to separate the majority of live beetles. The live adults should be weighed to determine their quantity. This metric is vital for maintaining optimal rearing densities, ensuring that sufficient numbers of live adults are available to support consistent egg production. As mentioned above, to maintain standardized adult populations, the weight of live adults should be adjusted to a target density, typically 250 g per crate. In order to achieve this quantity after the separation of live and dead adults, additional young sexually mature adults should be inserted

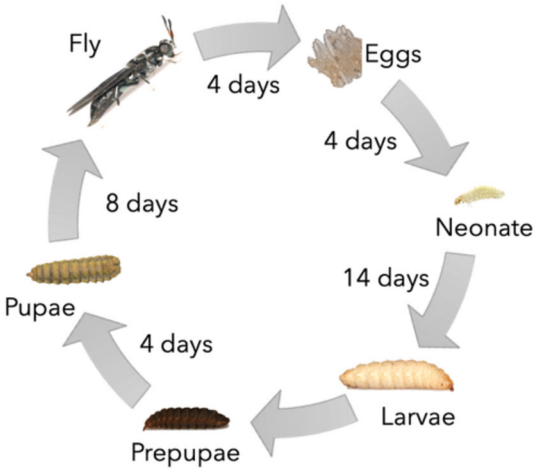

FIGURE 6 Life cycle of *Hermetia illucens*. The approximate time they are in each stage is indicated by the arrow next to their picture. The development time presented is at 30 °C for prepupae, pupae, flies and egg development, while at 27 °C (ambient temperature) for neonates and larvae and is further influenced by rearing conditions, nutrition and strain used.

and combined with the live adults collected. If enough new beetles have hatched it can be beneficial to replace more of the population (e.g. 25 or 33%) to ensure a fit and young population with a high reproductive capacity (Berggreen *et al.*, 2018; Frooninckx *et al.*, 2022; Morales-Ramos *et al.*, 2012).

3 Black soldier fly (*Hermetia illucens*)

Life cycle of the black soldier fly

The black soldier fly, *Hermetia illucens* (BSF), belongs to the order Diptera and family Stratiomyidae. Native to the Americas, BSF likely originated from South America, and has now achieved global distribution, mainly found in both tropical and warmer regions (Generalovic *et al.*, 2023; Kaya *et al.*, 2021; Ståhls *et al.*, 2020). BSF undergoes complete metamorphosis with four distinct life stages: eggs, larvae, pupae and adult (De Smet *et al.*, 2018) (Figure 6). Several studies have examined the life-cycle of BSF (Harnden and Tomberlin, 2016; Nguyen *et al.*, 2013, 2015; Oonincx *et al.*, 2015a; Tomberlin *et al.*, 2002; Zhou *et al.*, 2013).

The egg stage begins when the adult females deposit clusters of eggs near decaying organic matter, which provides proximity to a food source for neonates. Eggs are laid in dry small cracks or crevices, which provide protection and tend to hatch in approximately four days at 27 °C. The hatchlings are cream-coloured neonates which are less than 1 mm in length with a weight of 0.021 mg (recalculated from Deruytter *et al.*, 2024a,b).

The larval development time can be highly variable, ranging from less than two weeks to several months. Several factors can affect the larval development time such as temperature (Harnden and Tomberlin, 2016), moisture of the rearing substrate (Bekker *et al.*, 2021), relative humidity (Holmes *et al.*, 2012), substrate quality (Belperio *et al.*, 2024), population genetics and their interaction with environment (Greenwood *et al.*, 2021; Sandrock *et al.*, 2022), associated microbiota (Wynants *et al.*, 2019) and other factors. As the larvae reach their final larval stage they can individually easily weigh up to 220 mg (Lalander *et al.*, 2019) and develop a darker coloration, signifying their transition to the prepupal phase that uses the larval reserves to pupate.

The pupal stage follows, during which prepupae develop into pupae with a hardened exoskeleton. This stage can last 8 days or more, depending on the pupation substrate and other environmental conditions (Liu *et al.*, 2023). The pupae remain inactive while the interior undergoes a complete metamorphosis.

The final stage is the adult fly. Adult BSF are characterized by a slender black body measuring 15-20 mm in length, transparent abdominal windows, a single pair of iridescent wings and long antennae (Oliveira *et al.*, 2015). During this stage, the adults rely on the fat body reserves accrued during the larval stage for living. However, their lifespan can extend by feeding them (Bertinetti *et al.*, 2019; Bruno *et al.*, 2019; Nakamura *et al.*, 2016). During this period, adult BSF can form aggregations, known as lek, where the males compete for the females. Mating typically occurs two days after emergence. After mating, the females need two days to develop the eggs and oviposit (Lemke *et al.*, 2023). The female typically deposits only a single clutch of approximately 600-980 eggs in sheltered spaces, completing their reproductive cycle before dying (Barrett *et al.*, 2023a; Muraro *et al.*, 2024; Tomberlin *et al.*, 2002).

Infrastructure and resources

Given that the BSF is a holometabolous insect with a flying phase, the maintenance of its population needs two distinct modules/production units and resources tailored to its two main developmental phases (larvae and flies). These modules are:

- Rearing room: focused on feeding larvae until they reach the pupal phase
- Reproduction room: Dedicated to adult flies for mating and egg production.

Each of these two modules/stages has its requirements.

Rearing room

Similar to mealworms, maintaining a stock population of BSF can be done in any room that can store a number of crates and be kept at the required environmental conditions. The rearing temperature is arguably the most important environmental parameter. The impact of rearing temperature on the life cycle traits and the performance of black soldier fly larvae has been assessed in several studies (Chia *et al.*, 2018; Harnden and Tomberlin, 2016; Shumo *et al.*, 2019; Tomberlin *et al.*, 2009). However, it became clear that ambient temperatures do not represent the real temperature in which the larvae grow and develop (Bosch *et al.*, 2020; Gold *et al.*, 2020; Yakti *et al.*, 2022). Usually, when provided with a nutritious substrate and maintained at suitable larval density (i.e. >5 larvae/cm^2), the temperature in the substrate increases significantly compared to the ambient temperature. To ensure optimal conditions, ambient temperature should be regulated to achieve the desired higher temperature within the substrate. As the larvae grow and heat is generated in their rearing containers, the external thermal energy input can be gradually reduced. Nevertheless, ambient temperatures between 25 and 30 °C have been reported suitable in different BSF rearing systems (Chia *et al.*, 2018; Ribeiro *et al.*, 2022; Shumo *et al.*, 2019; Yuru *et al.*, 2018). Such ambient temperatures can lead to a substrate temperature of over 40 °C, depending on the rearing conditions (density, size of crates, height of the substrate, etc.) which influence the substrate temperature development. An increase in substrate temperature may be beneficial, as the optimal temperature for protease activity in the gut ranges between 45 and 47 °C (Bonelli *et al.*, 2019; Kim *et al.*, 2011). When the temperature exceeds the larvae's tolerance threshold, the larvae exhibit thermoregulating behaviour by dispersing at the top of the substrate and avoiding aggregation in the substrate (Yakti, unpublished work). Additionally, a temperature of 37 °C is shown to improve larvae survival upon infection with *Pseudomonas protegens* (Shah *et al.*, 2023). While not explicitly observed in BSF, a phenomenon known as behavioural fever, where collective behaviour raises the perceived temperature to combat infections, has been documented in other insect species, such as *Musca domestica* and *Schistocerca gregaria* (Elliot *et al.*, 2002; Watson *et al.*, 1993). To mitigate the risk of excessive heat generation, caused by high feed volume and high larvae density, it may be necessary to reduce larval density by redistributing the contents of a single crate into multiple crates.

In contrast to the temperature, the ambient relative humidity has a neglectable direct effect on the performance of BSFL in the presence of the moist feeding substrate. As long as the substrate is moist, the larvae would have high humidity in their vicinity and will perform well (Ewusie *et al.*, 2018). However, ambient humidity should be maintained at a level that prevents the substrate from drying out before consumed by the BSFL. A 60% RH is commonly used in BSFL trials and is considered suitable (Deruytter *et al.*, 2024a,b).

Besides the effect of temperature and humidity, addressing the carbon dioxide (CO_2) and ammonia concentration is important even though there is a lack of documentation on the direct influence of these gases on the development and growth of BSFL. Ammonia is a colourless, corrosive, and alkaline gas with a pungent odour commonly associated with organic decomposition in BSFL rearing systems. It was shown to be produced in varying quantities, associated with different substrate properties such as pH and protein content (Coudron *et al.*, 2024). Its odour is detectable at concentrations as low as 5 ppm but higher levels can lead to significant health risks. Moderate irritation to the eyes, nose, throat and chest has been reported at 50 ppm after 10 mins of exposure. Prolonged exposure to ammonia at concentrations >2500 ppm for 30 min or longer poses an acute risk to human health (National Research Council Committee on Acute Exposure Guideline, 2008). Therefore, it is important to maintain the levels that ensure safety for the facility/lab staff who maintain the population, which can be achieved by proper ventilation or an air exchange system. An active carbon-based air cleaning system can also be installed to clean the air from ammonia and volatile organic compounds (VOC's) characteristic to BSFL rearing.

Reproduction room

The reproduction room usually consists of smaller units, sometimes referred to as cages or fly stables. These cages serve as enclosure in which the flies are kept. The fly cages are designed to facilitate the mating and oviposition of adult flies (Figure 7).

The ambient conditions, such as temperature, relative humidity and lighting, in the reproduction unit play a crucial role for a successful mating and oviposition. The adult BSF exhibit a temperature preference which varies depending on the age and size, with older and smaller adults preferring cooler (≈19-21 °C), than younger and larger ones (≈27-29 °C) (Addeo *et al.*, 2022). However, temperatures lower than 15 °C and higher than 37 °C are considered unsuitable for egg pro-

FIGURE 7 SLU Uppsala, fly cages (BSF) adapted after design by EAWAG manual (Dortmans *et al.*, 2021). Water and egg traps are located on the outside of the cage, with the mesh allowing access to the water and egg traps while containing the flies.

duction, and a temperature of 30 °C has been described as optimal (Chia *et al.*, 2018). This temperature also allows the highest egg viability.

In addition to temperature, controlling humidity is essential for efficient BSF oviposition. Both temperature and humidity interact and should be controlled in parallel, as higher temperature would automatically reduce air relative humidity values when the same amount of air is present as a vapour in the air (Jaakkola, 2006). A relative humidity level between 60-70% is considered suitable (Macavei *et al.*, 2020; Tomberlin and Sheppard, 2002) and has been frequently used in BSF oviposition studies. A relative humidity in this range also allows high adult emergence and egg hatching, in addition to short development time from larvae until adult emergence (Holmes *et al.*, 2012).

Besides an adequate temperature and humidity, the adult flies require light for successful mating. Hoc *et al.* (2019) observed that egg yield increased logarithmically with longer daily light durations. Although a direct comparison between 6 hours of light and 16 hours of light revealed no significant difference. However, Liu *et al.* (2022) found that increasing photoperiod from 8:16 to 16:8 (light:dark) increased neonate production. If an artificial light is to be installed in the reproduction room, selecting the right light source in terms of wavelength, intensity, and setting the right photoperiod is crucial for maximizing egg production. The egg hatchability and oviposition are usually preserved when halogen or quartz-iodine lamps are used instead of sunlight (Singh *et al.*, 2023; Zhang *et al.*, 2010). However, the ommatidia of BSF contain photoreceptor cells sensitive to ultraviolet (UV), blue and green light (Oonincx *et al.*, 2015a). LEDs that produce this light spectrum seem to be superior to other artificial lights (Macavei *et al.*, 2020). An LED intensity of at least 200 μmol/m² per s has been suggested as a minimum requirement for mating, and achieved 75% of mating observed under the sun (Nakamura *et al.*, 2016), while in another study, a 40 μmol/m² per s was sufficient for mating (Hoc *et al.*, 2019). For quartz-lamp, an intensity of 135 μmol/m² per s has been found optimal, while a 160 μmol/m² per s light intensity produced by rare-earth lamp did not allow any mating (Zhang *et al.*, 2010). It is important to note that different light sources can also emit thermal energy to different extents, which exposes the flies to higher temperatures and reduces the relative humidity. Additionally, the different light spectra investigated make intensity measurements challenging and inconsistent throughout the literature making it challenging to conclude an optimal light intensity (Athanassiou *et al.*, 2025). Lastly, the genetic background of the flies may also play a role, as demonstrated by Liu *et al.* (2020). Their study tested two different BSF strains under four types of artificial light, revealing significant differences in egg production between the strains.

When the objective of a laboratory is to maintain the breeding population rather than maximise the output,

it might be feasible to relax strict control of ambient conditions, as the flies thrive in a range that is not their ideal condition. Hence, the fly cages may be placed in greenhouses that can vary in environmental conditions (Sheppard *et al.*, 2002). This can save on costs for lighting but increase the cost for heating or cooling in some areas. In contrast, an indoor system offers the advantage of climate and light control, leading to a stable and predictable egg production but results in higher energy demand.

Finally, usually the flies are contained inside fly cages/stables. Cage dimensions can vary greatly with Nakamura *et al.* (2016) using cages of only 0.02 m^3 (27 × 27 × 27 cm) while Sheppard *et al.* (2002) used a cage of 16 m^3 (200 × 200 × 400 cm). These come in different setups but can mostly be divided into "soft" cages and "hard" cages. Soft cages are primarily made from loosely woven fabrics, such as insect netting, sewn together. This setup is flexible, allowing for easy assembly and disassembly. The entire cage can be sanitized by hand or in a standard laundry machine. On small scale, e.g.: laboratory scale or small-scale farming, this setup is easily scalable by adding more sewn cages on demand. Egg traps and water can be placed inside or outside the cage, with the latter set-up efficiently containing the flies, as egg collection or water maintenance can be performed without reaching into the cage. When not operated in a greenhouse, lights, water and ventilation are usually not part of the soft cages but rather fixed within the building structure. Hard cages are constructed from rigid materials such as glass, plexiglass, metal, wood, mesh or similar. The hard cages cannot typically be washed as a whole, instead they require manual cleaning. The lighting, ventilation, water supply and egg traps are typically integrated in each cage and are usually not transferred between cages. Maintenance and egg collection can be located outside or inside the cages with the latter requiring personnel to enter the fly cage. Different materials such as metal vs wood might store and release temperature differently, possibly creating microclimates for flies (Addeo *et al.*, 2022). Moreover, a larger volume can create different light or temperature conditions within the cage, hence influencing the fly's behaviour (Liu *et al.*, 2022).

Feed requirements

BSF larvae are voracious feeders, and combined with the fact that the species can grow and thrive on a wide variety of substrates makes it among the most important insects produced for food and feed (Fitriana *et al.*, 2022). For population maintenance, it is important to provide the larvae with a sufficient amount of a high-quality substrate to achieve a high egg production per adult (Laursen *et al.*, 2024). Their nutrient requirements are discussed in more detail in Oonincx *et al.* (2025). In many laboratories, stock populations are typically provided with grain-based feed, though the specific composition often varies between facilities (Deruytter *et al.*, 2024a,b). A dry feed, such as chickenfeed, can simply be mixed with water to achieve approximately 30% dry matter content. If the water-holding capacity of the grain-based feed is low and free water was formed in the feed, high fibre components (e.g. blended straw or wheat bran) can be added to bind the free water and improve the physical structure (Yakti *et al.*, 2023).

The fly stage is the polar opposite of the larvae stage as they have minimal feed requirements. The impact of supplying water (Sheppard *et al.*, 2002) and nutrient-enriched water (Bertinetti *et al.*, 2019; Chia *et al.*, 2018; Klüber *et al.*, 2023; Macavei *et al.*, 2020; Nakamura *et al.*, 2016; Thinn and Kainoh, 2022) on several reproduction parameters (longevity, egg mass, hatchability) has been investigated. The results of these studies are ambiguous. While Nakamura *et al.* (2016) found that longevity improved significantly when providing sugar and distilled water, no egg parameters were monitored. Bertinetti *et al.* (2019) saw that the amount of eggs was 3 times higher when providing a protein source (artificial milk) compared to a treatment receiving water. Macavei *et al.* (2020) demonstrated that providing sugar and water significantly improved egg production compared to supplying only water or no supplementation. Klüber *et al.* (2023) found that while a glucose- or protein-rich microalgae solution did not enhance egg production compared to tap water, a 5% honey solution led to an 80% increase. Finally, Chia *et al.* (2018) reported no clear link between providing a 10% sucrose solution and higher egg production, despite observing improved adult longevity. These results indicate that supplying sugar, water and a protein source may positively affect reproduction parameters. Although it is likely that the source of protein and sugar used codetermines the success rate of supplying nutrients to the flies. Note that maximising egg yield is not strictly necessary when just maintaining a population and supplying nothing or only water is sufficient for basal egg production, although the argument could be made that withholding water from the flies could be seen as an infringement on their welfare.

Good practices per stage

Mating, eggs and neonates

Provided that fresh, healthy flies are kept at the correct environmental conditions with adequate light they should mate and deposit their eggs within a week. The amount of flies that is desirable in a cage is up for debate with literature describing a range of 3700-6500 flies/m^3 (Hoc *et al.*, 2019; Liu *et al.*, 2022), some even claiming up to 20 000 flies/m^3 (Tomberlin *et al.*, 2025). Even among the authors there is ambiguity, with densities ranging from 7000 flies in an 80 × 110 × 135 cm cage (or 5610 flies/m^3) to 3000 flies in a 60 × 60 × 90 cm cage (or 9260 flies/m^3). Nevertheless, several thousand flies per m^3 should yield sufficient eggs to maintain your population. The exact number of eclosed flies is typically not determined, except perhaps in high-tech facilities or with small populations. Instead, it is often estimated based on the amount of pupae and their average weight. It can be assumed that the eclosion rate is close to 100% when stored under optimal conditions, as unpublished data from our own research indicates. A desired amount of prepupae or pupae (and corresponding number of flies) can be placed directly in the cage. However, following fly eclosion the presence of pupae exuviae inside the cage may undesirably lure flies to oviposit there, subsequently leading to misplaced eggs. Alternatively, they can also be placed in an adjoining dark cage, this may be a light-proof box, fabric enclosure, or a darkened room (Dortmans *et al.*, 2021). There are two main strategies on how to maintain a fly population batch vs continuous:

- Continuous release: This strategy aims to maintain a fly population over a period that exceeds the lifespan of the first batch that was originally introduced by continuously adding new batches of fresh (pre)pupae. This can lead to a mix of generations, but should result in a continuous availability of sexually active flies (although potentially with variations in the density). It is important to regularly remove dead flies which will accumulate, to prevent potential disease spread or the attraction of insects (BSF or other pest species) that may lay eggs. Keep in mind that flies of different ages often exhibit distinct temperature preferences (Addeo *et al.*, 2022), and males may develop faster than females (Hoc *et al.*, 2019).
- Batch release: This all-in, all-out strategy, introduces a batch of (pre)pupae at a similar developmental stage, with the aim to synchronize egg-laying, which simplifies cleaning. The cage is typically run for a set period, with oviposition peaking around day 4 after release. The disadvantage of this method is that the flies can emerge at different time points, sometimes with days in between the first and the last fly. This can impact the availability of mating partners within a batch, potentially altering the egg yield per cage.

To harmonize egg production, there is the option to delay mating by keeping the cages dark or using a dark cage. While flies still eclose from their pupae while kept in the dark, they further remain inactive and wait for a light stimulus to mate and oviposit. By pressing this "pause button" the majority of flies has had the time to eclose and finish maturation by the time the light is turned on or they are introduced in the love cage. This way, depending on variation in pupation development, up to six egg harvests (one every two days), can be reduced to just one to three, resulting in fewer larger egg harvests rather than more smaller ones. Own unpublished research has shown that flies can be kept in the dark for up to one week, but a loss of productivity and flies is to be expected.

It should be noted however, that this comes at an underestimated risk. With batch production systems with discrete remounting there is the possibility for genetic substructures reinforced by random genetic drift among temporally staggered and thus genetically more or less isolated breeding batches. Such unintended outcomes may at worst affect phenotypes, and consequently the performance of control treatments over time.

When providing water to the flies, this can be done with a partially submerged structure that sucks water through pores to the surface (e.g. sponge, cotton, cloth, etc.) or with a netting spread over the surface of a water body (Figure 7), preventing flies from falling in and drowning. The latter with the disadvantage that dropping water levels due to evaporation, may place the water out of the flies' reach. An alternative may be a perforated piece of polyethylene that floats on a water surface and circumvents the problem of dropping water levels (Figure 8). Providing nutrients to the flies can be done by dissolving them in the water but comes at a risk of creating a growth medium for undesirable microbiota. In some cases, the water source is completely removed once oviposition has started, to avoid microbiota to grow and eggs being misplaced. Alternatively, water can be applied to surfaces (netting, walls or other structures inside the cage) by spraying or misting (Sheppard *et al.*, 2002). The downside of this method is a potential growth of microbiome, such as fungi, bacteria or algae, on the constantly moist surface.

Typically, oviposition and egg collection take place in a centralized ovitrap (oviposition or egg trap), a specialized structure designed for egg-laying. Female

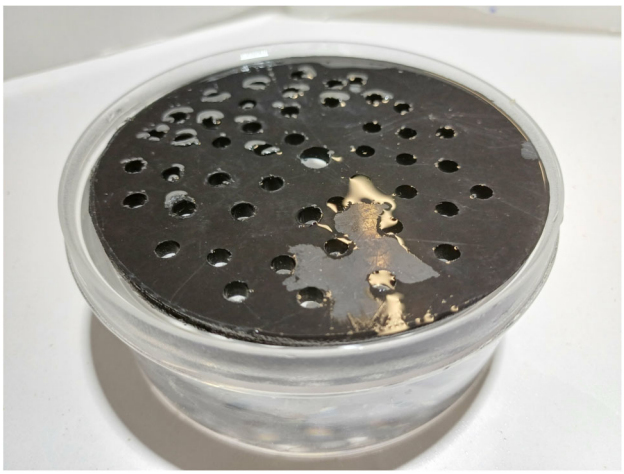

FIGURE 8 Water supply for BSF adults with a floating perforated polyethylene piece.

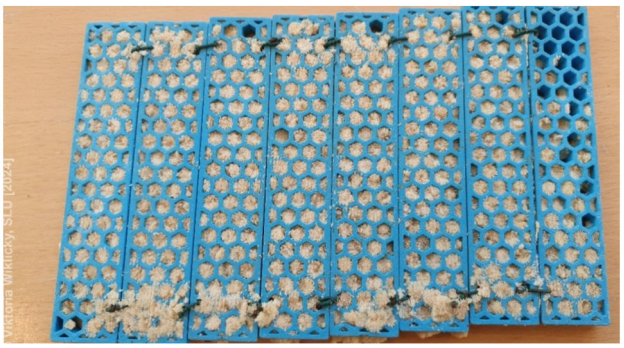

FIGURE 9 Egg trap at SLU (Uppsala) made from sustainable materials, durable and suitable for regular dishwasher use (bioblo.com).

flies are drawn to the ovitrap, which features multiple dry crevices that provide ideal sites for egg deposition (Sheppard *et al.*, 2002). Drawing in the flies is often done with a lure (also called attractant). Which is usually underneath the actual ovitrap to prevent flies from drowning in the lure. Various studies have used a plethora of different lures, e.g. Dortmans *et al.* (2021) suggest a mixture of water, frass and dead flies. However, no optimal recipe is set in stone, except for one common theme: it should be smelly. In recent years, more attention is drawn to which compounds trigger a female fly to choose a particular spot as an oviposition site (Klüber *et al.*, 2024; Piersanti *et al.*, 2024; Thomas *et al.*, 2024). This would be beneficial as it would take away a source of variation due to differences in composition as well as a source of potential contamination as the organic lure are typically subject to microbial decay.

As for the actual ovitrap, although various studies performed experiments in order to determine the preferred materials of female flies to oviposit upon, with wood or cardboard performing better than plastic traps (Julita *et al.*, 2021), own experience has shown that although not the preferred spot when given the choice, plastic traps or other plastic-like material work equally well when no other options are available (Figure 9). This has the merit that plastic does not absorb atmospheric moisture, is therefore very stable in weight, and can be used more easily to determine egg yield without the need to manually remove the eggs from the ovitrap, avoiding the risk of damaging eggs in the process. There are a variety of commercial ovitrap designs available.

Eggs are best harvested before they have the chance to hatch when still inside the cage. We therefore recommend to harvest eggs at least every two days. A single egg approximately weighs 0.028 mg (Macavei *et al.*, 2020). Combining data from different papers mentioned above means one female is expected to lay an egg clutch of around 16.8 mg (assuming 600 eggs), using the priorly stated 3700 flies per m^3, and assuming a 1:1 female-to-male ratio, a 1 m^3 cage is expected to produce at least 30 grams of eggs. Eggs can be hatched directly above their feed, typically consisting of chicken feed (70% moisture content) (Dortmans *et al.*, 2021) or on Gainesville diet as described by Deruytter *et al.* (2024a,b) (comprised of 67% water, 16.5% wheat bran, 9.9% alfalfa, 6.6% corn as is; Hogsette, 1992), in a so called hatchling shower. Although this may introduce a source of variation as not all neonates hatch simultaneously. It is therefore recommended to replace the feed under the hatchling shower at set intervals (24 h). Another disadvantage is that the exact amount of neonates that has hatched is unknown and the density of larvae may therefore vary. Causing more variation in the starting weight when grow-out starts. Hatching neonates in a sealed bucket (with airholes covered with fine netting to prevent escape) before introducing them on their feed is an alternative. The neonates are initially deprived of feed (no longer than 24 hours), meaning that early hatchlings cannot make a head start. However, they can be weighed and the feeding amount can be better adjusted to the number of larvae. This reduces the amount of variation between individual larvae and different batches.

Alternatively, In certain parts of the world, it is also possible to use wild caught/lured eggs or larvae. This is not a straightforward endeavour (Ewusie *et al.*, 2019), yet might provide advantages of local genetic ecological adaptation in semi-open conditions (Julita *et al.*, 2020; Kaya *et al.*, 2021). However, there may also be disadvantages, such as natural parasites (Ewusie *et al.*, 2019) or a lack of adaptation to largely artificial conditions possibly resulting in failure to establish (Tomberlin

et al., 2025) or subsequent genetic bottlenecks or even population collapse (Rhode *et al.*, 2020). Furthermore, wild catches pose a challenge of insecurity of availability and attracting other insect species. It requires less lab space to not have a constant fly population running, yet at the cost of stronger dependencies on seasonality (Ewusie *et al.*, 2019). Moreover, predictability may be compromised because depending on the type of feed provided, wild-type BSF may exhibit higher within-batch phenotypic variation regarding key developmental and performance traits (Zhang *et al.*, 2024), possibly owing to their generally more diverse genetic background compared to globally common domesticated strains (Kaya *et al.*, 2021). Lab reared flies are better adapted to controlled conditions, these are consistently available, and are reported to better perform in waste conversion (Zhang *et al.*, 2024). Maintaining a lab population requires space, equipment, and expertise.

Rearing instars

Larvae rearing is typically done in two stages. Initially, neonates are nursed for 5 to 7 days on a starter feed (e.g. chicken feed or Gainesville diet) at a higher density. Deruytter *et al.* (2024a) proposed 1 g of neonate larvae per kg of Gainesville diet, which is expected to yield 48 000 larvae. This is followed by a second rearing phase, sometimes called "fattening stage" or "grow-out". At the beginning of this phase larvae are typically redistributed to achieve a lower density. A desired number of individuals are placed in rearing crates, typically measuring around 60 × 40 cm. The amount of feed provided must be adjusted based on the substrate used and on the purpose of the larvae, whether it is experimental usage or maintaining the population.

A common practice is to start with a batch of larvae of approximately similar size. Some literature refer to these as "5-day-old-larvae" (5DOL) but due to the plasticity in insect larvae growth rates, a more accurate method is to report the average weight of starter larvae in mg or report head capsule width as this can differentiate between instars of a BSF larvae (Barros *et al.*, 2019). Estimating the amount of 5DOL is not strictly necessary when larvae are used solely for maintaining the population, but it remains a useful tool for standardization as it assures accurate feed allocation per larvae. To estimate the number of 5DOL, a sampling method as was proposed by Dortmans *et al.* (2017) can be used. This option is preferred over correlating the weight of the initial egg cluster to the number of larvae, as egg hatch rate and larvae survival can vary depending on external factors, as stated in the section Mating, eggs and neonates.

For sampling, gently but thoroughly mix the batch of 5DOL to ensure uniformity. Collect three subsamples, and count the number of larvae in each sample. The BugBook article by Deruytter *et al.* (2025) discusses the appropriate subsample size, emphasizing that using over 100 larvae per sample is generally recommended to ensure accuracy and reliability in results. Calculate the average number of larvae per gram based on these counts. This allows for an estimation of the total number of larvae in the batch by extrapolating from its overall weight. These larvae could be used in a feeding experiment, which is elaborated on extensively in the BugBook article by Deruytter *et al.* (2025). However, a part of these larvae should be used to replenish your own population of adults. We recommend to keep at least 2% and a minimal effective population size of 100 individuals (discussed in more detail below in the section Maintaining different insect strains).

During the grow-out, the larvae can be left undisturbed. Intermediate feedings are not strictly necessary when sufficient feed was present at the start. The larvae will gradually turn into prepupae after at least one week, although this may take (a lot) longer depending on feed properties and environment. The end of the larval grow-out phase is marked by the appearance of prepupae and cooling and drying of their substrate. At this stage, it is common practice to separate larvae or prepupae from their frass. Various methods are employed in laboratory settings, with the literature describing techniques such as dry sieving, hand picking, rinsing, passive separation and self-harvesting:

- Dry sieving: This method is the most convenient. It involves actively sieving the end product to separate the smaller (and dry) frass particles from the larvae. Mesh sizes that can be used can vary depending on larvae size and frass particle size, typically 2 to 4 mm mesh sizes are employed.
- Passive separation: The substrate and larvae can be placed on a sieve, allowing the larvae to crawl through, leaving the substrate behind. Alternatively, the larvae and frass can be placed in a spacious crate, the larvae tend to cluster together and can be manually extracted. This method is often used with substrates with large or wet and adhesive frass particles. Separation between larvae and frass will not be perfect.
- Self-harvesting: Prepupae often disperse from their feeding grounds in search of less crowded sites to pupate. This behaviour can be exploited by encouraging their escape, a process that can be facilitated by wetting the frass in the crates.

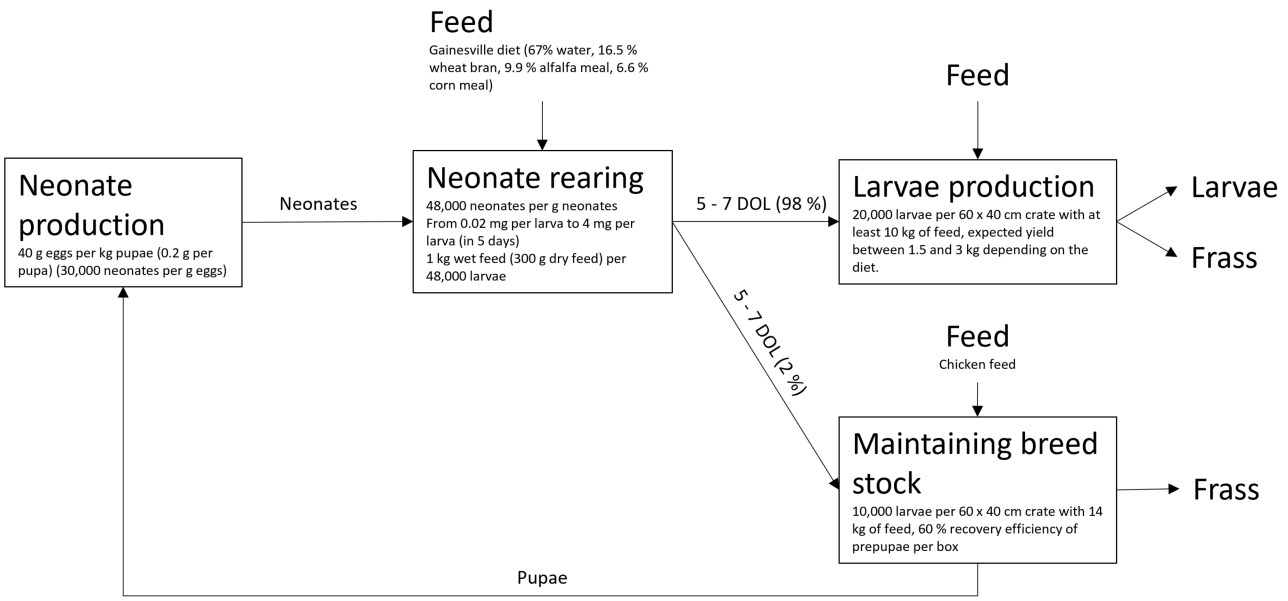

FIGURE 10 Example of a BSF maintenance flow employed at Inagro. DOL, day-old-larvae.

- Active wet separation or rinsing separation: Larvae are flushed out by washing away the substrate. A drawback of this method is the production of large volumes of eutrophied water.
- Hand separation: Larvae are manually separated from the frass by picking out individual larvae. This is primarily used on a small scale for research.

New adults

Prepupae can pupate in substrates such as wood shavings, vermiculite, potting soil, frass, or dried frass, providing a medium for burrowing during metamorphosis (Dzepe *et al.*, 2020). However, reusing pupation material necessitates separating empty pupal shells, which can be labour-intensive. After separating out the frass, prepupa or pupa can also be left bare during their time of metamorphosis, reducing material handling and sanitation efforts. However, this may require optimizing density and conditions to ensure successful development.

To better manage production, it may be beneficial to have practices in place that allow the delay of certain steps in the process: for instance, when flies are expected to emerge on a Wednesday, eggs would be available in the weekend when no caretaker is present. Experience from multiple BSF facilities shows that prepupa or even pupae can be stored below optimal temperatures (12-18 °C), which helps delay development. The duration of cold storage can be several weeks, with durations longer than 5 weeks impacting negatively on the egg production. However, note that the specific response of your population to these temperatures may depend on your specific BSF strain.

In BSF production, nothing is set in stone. At each step of its life cycle different BSF producers, whether it is experimentally or professionally, have their own intricacies on how to handle, rear and reproduce these insects. Despite that, the authors want to give some concrete handholds for starters into this endeavour. Figure 10 shows the flow of a production process, including quantities of eggs, neonates, larvae and feed used in each step.

4 Additional considerations when maintaining insect populations

Allergenicity

A risk that cannot be overlooked is developing an allergy related to the insects reared. Anecdotally, the authors know of several people that needed to quit insect related activities due to an allergic reaction worsening over time. The risk of becoming allergic is reaffirmed in a study by Ganseman *et al.* (2023) that tested 15 employees who were regularly exposed to i.e. mealworms and black soldier fly. 60% of the test subjects reported symptoms to the upper respiratory tract (sneezing, blocked/runny nose, itchy nose/mouth/throat) when operating the insects. 58.3% showed positive reactions to skin and blood tests. It is possible that an allergen from one insect species (or other invertebrates such as house dust mite) leads to an allergic reaction by similar allergens in other insect species, also known as a cross-reaction. However, co-sensitization is also likely,

in which allergies are caused by unrelated allergens between species (Ganseman *et al*., 2022).

Although allergies to both mealworms and black soldier fly are possible, cases are more reported in mealworms. Allergens are mostly dispersed via dust particles, by which they can come into contact with skin, mucous membranes in the upper respiratory tract and the eyes. Dust production is more severe in mealworm production due to the high dry matter content of its feed and frass, especially during activities where frass becomes airborne (e.g. during sieving). The authors therefore recommend to protect oneself against the dust, especially, but not exclusively with mealworms. Prevent inhalation of dust particles and contact with the skin. Because exposure is highest while sieving, it is recommended to carry out all sieving activities in a therefore designated room and enter this room only while fully protected by a full face mask with air filtration (FFP3) in combination with a disposable coverall and gloves. This offers sufficient protection against most allergens. Dry cleaning (blowing of equipment with compressed air, sweeping) should be avoided as this makes allergens airborne again, instead clean using water or a vacuum cleaner (with appropriate filter).

Pests, diseases and toxins

There is still inadequate information towards the problems and risks that are related with the occurrence of pests and diseases that may affect insect rearing. For a mealworm population, a recent review by Deruytter *et al*. (2021b) indicated that infestations by stored product moths of the family Pyralidae, such as the Indian meal month, *Plodia interpunctella* (Hübner) and the Mediterranean flour moth, *Ephestia kuehniella* Zeller is common. However, other species that may occur in insect farming are stored product beetles, such as the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), as well as stored product mites, such as the meal mite *Acarus siro* (Acari: Acaridae) (Athanassiou and Buchelos, 2001; Athanassiou *et al*., 2001; Hagstrum and Subramanyam, 2009). In BSF rearing, the main problems arise with different fly species, most notably the house fly (*Musca domestica*) and fruit fly (*Drosophila* sp.). Finally, also rodents are known to make their way into an insect production to feast upon the insects.

The infestation patterns of these species and the damage or problems that they can cause in laboratory or mass rearing are not clarified in detail in terms of the actual damage estimates. It is well-known that certain species, such as *T. castaneum*, predate other species, especially on their eggs (Nansen *et al*., 2009), which is expected to reduce the offspring when coexisting in the same substrate. Nevertheless, there are no quantified data of the actual loss in insect production, directly, through their effects on the farmed species, and indirectly, through the consumption of the raw materials that are used for insect rearing. Unfortunately, as in stored product facilities, co-occurrence of different pest species at the same time is possible (Athanassiou and Buchelos, 2020; Nansen *et al*., 2009). Although the presence of other arthropods in the insect farming chain is a definite safety issue, and should be regarded as such, so far, there are no established sampling protocols to estimate these risks.

The source of the infestation is mainly through the import of raw materials or via improper confinement methods (e.g. open door). The control measures that should be taken for pest control in the case of a typical storage and processing facility, such as a pasta factory or a flour mill, can be directed directly towards the target pests, without affecting the final product (Athanassiou and Arthur, 2018). On the other hand, in the case of insect farming, most typical control measures will affect the farmed species and as such, most of the major measures that are currently in use should be avoided. There are three steps that have to be followed to control insect infestation in insect farming: prevention, monitoring and control (Athanassiou and Arthur, 2018). Prevention is related with all the efforts that are considered necessary to make the building "insect proof", minimizing insects that come from outside, or live insects that can escape from the production chain, which could become a major source of new biological invasions (Bang and Courchamp, 2021). This means that openings (large gaps in doors, windows, etc.) should be avoided, and raw materials that are used for the production of insects should be carefully inspected through a thorough "gatekeeping" control upon receival (Stejskal, 2015). However, the most important measure that has to be taken at that stage is sanitation, in order to avoid insect refugia that will allow pest reproduction and further dispersion (Morrison III *et al*., 2019). In this context, even the slightest occurrence of product residues and debris can host large number of stored product insects, that will eventually continue to cause damage in the raw materials (Bingham and Hagstrum, 2023). Avoidance of these infestation foci constitutes the cornerstone of prevention at the post-harvest stages of durable agricultural commodities.

As the next step, monitoring should be established from the very beginning and throughout. There are different types of trapping devices that can be used, while the decision for the selection of these types is based on the target pest that is to be monitored. For instance, for flying insects, such as Pyralidae, pheromone-baited aerial traps with adhesive surfaces are used, while for crawling insects, such as most stored product beetle species, the usual type are so called "floor traps", that can be effective even without bait (Athanassiou and Arthur, 2018). Sampling is also a critical step in detection and estimation of the presence of insects and has to be incorporated in monitoring protocols, especially in the case of the raw materials that are received in the facility (Athanassiou and Buchelos, 2001; Athanassiou and Arthur, 2018). However, there are no established thresholds so far for decision-making, which is a serious gap that has to be further evaluated.

The application of control measures in insect farming is probably even more complex than prevention and monitoring, for reasons that have been already mentioned. Fumigation with aerial insecticides can be used only in the case of raw materials and only in designated areas, e.g. in silos and horizontal warehouses, that are away from the insect rearing, given that leaks may also be lethal for the latter. The same holds in the case of the application of contact insecticides that are applied as "fabric" treatments or in "crack and crevices". Nonetheless, insecticidal residues from previous applications in the raw materials may also affect insect rearing. There are some methods, however, that can be used with success for the control of pests with little or no effect on the farmed insects. One paradigm is the use of diatomaceous earths, that has been proven effective for the control of different pests, but they do not affect larvae of *T. molitor* (Gourgouta *et al.*, 2022). Other methods can include species-specific parasitoids, that are effective for the control of certain species, with no adverse effects in the mass rearing units (Athanassiou and Arthur, 2018). Still, the augmentative release of parasitoids may increase the presence of insect-related contaminants into the final product, and should be regarded in combination with other control measures. Other methods include mass trapping through specialized trapping devices, extreme temperatures, and mating disruption (Athanassiou and Arthur, 2018; Deruytter *et al.*, 2021b), but there is still inadequate information on how these techniques can be adapted in insect farming.

Besides pest species, farmed insects may also be exposed to diseases. Some studies have assessed the potential risks of disease occurrence, but they are still insufficient for quantitatively evaluating the extent of the damage these diseases can cause. Some of the risks may be related with qualitative characteristics of the raw materials, which underline the need of quality control, as it is done in the case of other livestock production chains. Moreover, there are some pathogens that affect the insects themselves and may pose a serious risk in insect farming, such as entomopathogenic fungi, bacteria and viruses (Joosten *et al.*, 2020; Klüber *et al.*, 2022; Pienaar *et al.*, 2022). For instance, Jensen and Lecocq (2024) reported a series of diseases that may affect *H. illucens*, along with the potential challenges in insect production. Indicatively, She *et al.* (2023) reported a series of potentially pathogenic bacteria that were isolated from *H. illucens*, while Lecocq *et al.* (2021) found that this species is susceptible to the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypecreales: Cordycipitaceae), which is a common insect pathogen in stored product insects (Rumbos and Athanassiou, 2017). Similar studies have been reported in the case of *T. molitor* (Eski and Gezgin, 2022; Pöllinger-Zierler *et al.*, 2023). Indirectly, several stored product insects can introduce non food-borne pathogens, such as enterococci (Parlapani *et al.*, 2020).

Finally, farmed insects may also be exposed to contaminants in their diets, such as pesticides, heavy metals or mycotoxins. Despite the welcome role of insects as bioremediation agent in potentially tricky side-streams, sometimes observed substantial impairment during development (Heuel *et al.*, 2023; Meijer *et al.*, 2021; Niermans *et al.*, 2021) is alarming. Synergistic effects upon exposure to multiple compounds (Meijer *et al.*, 2024) indicates there may be similar tipping points upon combined exposure of toxins and pathogens, as observed e.g. in pollinators (Fauser-Misslin *et al.*, 2014). Additionally, certain levels of these contaminants will be present in the final product.

Maintaining different insect strains

When maintaining different strains of an insect species, whether it be mealworms or black soldier flies, it is crucial to avoid cross contamination between these strains to preserve pure populations. Often there are no clear phenotypic differences between strains and as such when cross contamination occurs, it may long go unnoticed, meanwhile undoing all breeding efforts that have been done so far. The hazard is real and can be caused by the smallest moment of inadvertence.

For *T. molitor* there is a hazard of accidentally mixing the content of two crates (beetles or mealworms)

together due to inattention or unclear labelling. During sieving when removing frass, the mealworms can get stuck or cling onto the sieve unnoticed and as such be introduced in other populations when using that sieve moments later for other populations. Similarly beetles can cling onto a sieve as well and egg sieves used across strains may still carry residual eggs. In BSF, larvae could potentially escape from their crates and fall into nearby crates (which could be from another strain). Flies could escape and haphazardly make their way into another cage. Even tools, such as knives used for egg harvesting, can transfer eggs if not properly cleaned between uses. The list of potential hazards is endless.

Ideally, different insect strains should be maintained at separate locations, with distinct sets of instruments designated for handling each strain. However, this may not always be feasible due to resource constraints. As an alternative, using colour-coded crates and instruments for specific strains can serve as an effective visual cue to keep them separated. Furthermore, caretakers should prioritize maintaining a clean work environment and are advised to wear disposable gloves and cloth coveralls to minimize the risk of cross-contamination between strains.

Minimal population size – introducing new genetics

From a population genetics and breeding perspective, the initial establishment as well as continuous management of a population are of key importance. The above mentioned set up of wild populations exemplifies the possible risk associated with initially limited standing genetic variation to allow a given population to adapt to a new environment, i.e. a farming context, and further mitigate inbreeding in the long term. Particularly in the BSF, cases of rapid population collapses have been documented (Rhode *et al.*, 2020), and our understanding of the role of random genetic drift and selective pressures on genetic diversity in artificial regimes is improving (Hull *et al.*, 2024). However, inbreeding may be naturally promoted by positive assortative mating (Hoffmann *et al.*, 2021), and by no means necessarily results in population crashes (Cai *et al.*, 2022). Insofar, genetic health of a population can be considered context dependent. A pre-adapted production strain tends to be at lower risk to fail in another artificial setting than a local wild population, likely explaining how a decisive genetic bottleneck associated with domestication (Generalovic *et al.*, 2023) promoted homogenising the population genetic landscape across global BSF farms by a single strain (Kaya *et al.*, 2021). Further exploring the phenotypic variation within (Gligorescu *et al.*, 2023) and across genetically distinct populations (Sandrock *et al.*, 2022) will inform dedicated selective breeding (Hansen *et al.*, 2024) and reveal putative trade-offs between production-relevant performance traits and fitness (Hull *et al.*, 2024).

Nevertheless, regardless of the motivation, be it rearing or breeding, the basic setting as well as the specific management represent a selective regime that contributes to shaping genetic composition of future generations. Therefore, even basic rearing should mitigate unnecessary decreasing genetic diversity due to chance events. This can happen when the number of effectively reproducing individuals is low, rendering allele frequencies subject to stochasticity and increasing risk to lose rare variants. If such variants were yet essential to combat a future pathogen challenge, low effective population sizes will continuously decrease the adaptive potential of a given population.

From an applied perspective, as a rule of thumb, at least 100 individuals should contribute to the next generation (the more the merrier) (Frankham *et al.*, 2014). This means that routine maintenance for remounting the breeding stock needs to account for the sum of all practically relevant steps affecting this target. For instance, the collection of eggs on a single occasion during the cycle should not only ensure respective numbers of egg clutches are available during this critical step, but should at the same time account for additional downstream bottlenecks, such as synchronizing neonatal age-cohorts via hatchling showers and so forth. Additionally, to maintain genetic diversity, eggs should be collected from multiple mothers to ensure a broad genetic base in the population. A single egg clutch is typically from a single female, so different egg clutches likely have different maternal origins. The effective population size, i.e. the number of parents contributing to a given population can be readily inferred based on genotyping (Kaya *et al.*, 2021; Sandrock *et al.*, 2022).

Moreover, insect phenotypic plasticity may include transgenerational effects, which means that the performance of a given generation can be impacted by the environment that its parents faced (Boatta *et al.*, 2024; Gligorescu *et al.*, 2023). Accordingly, the standard rearing diet will inevitably influence the outcome of a test diet in the absence of an adaptation, and should thus be carefully chosen to either be or be not part of the experiment in form of a control diet. In this sense, particularly the comparison of different strains as an experimental factor is highly recommended to consider acclimatisation on such standard regimes preceding an experiment for one but preferably more generations (Sandrock *et*

al., 2022), e.g. to avoid results are merely influenced by "home effects" otherwise challenging conclusions (Zhou *et al.*, 2013).

Finally, in order to help disentangling effects of environments such as diets and genotypes, as well as the relevance of their interactions, more widely implemented basic genotypic characterisations of strains used for research would be valuable. More information on the rationales and tools relevant for molecular, population, quantitative and functional genetics are detailed in depth in the corresponding BugBook article by Sandrock *et al.* (2025).

Insect welfare

When maintaining mealworm or BSF populations for experimental purposes, it is recommended to consider insect welfare. Although there is no scientific consensus on what constitutes good welfare practices for these species, the topic is gaining attention among researchers and has been discussed for BSF by Barrett *et al.* (2023a) and for mealworms by Barrett *et al.* (2023b). These authors suggest adopting Brambell's Five Freedoms as a framework for insect welfare. This entails freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury, and disease, freedom to express normal behaviour, and freedom from fear and distress. The aim of these principles is to ensure that animals are kept in conditions that support both physical health and behavioural integrity. To what extent these freedoms apply to insects and how insects perceive them remains a subject of ongoing debate.

Nevertheless, maintaining a healthy and stable insect population requires that physiological and behavioural needs are met. Insects require appropriate environmental conditions (temperature, light and ventilation). They need water and the right amount of nutrients. Insect housing units and the number of individuals per unit should be managed in a way that prevents issues with overcrowding, such as cannibalism, overheating and diseases. These husbandry aspects are addressed in detail throughout this manuscript. While insect welfare is not the central focus, the practices described are largely consistent with the Five Freedoms framework.

Certain procedures, however, warrant additional ethical consideration. The culling of surplus populations should be conducted using methods that minimise suffering; freezing, maceration, and blanching have been proposed as acceptable approaches. Moreover, any experimental protocols involving starvation, crowding, or exposure to harmful agents should be clearly justified on scientific grounds and take into account the three R's (replacement, reduction, refinement). These were originally formulated by Russell *et al.* (1959) to guide the ethical use of animals in research. These principles encourage researchers to replace animals with alternatives where possible, reduce the number of animals used, and refine procedures to minimise pain, suffering, or distress.

5 Publication information

When preparing a publication on insect experiments, we urge to provide the following information about the insects used. Provide the scientific name of the insect species, including the strain or subspecies. Include details about the origin of the stock population (e.g. wild collection, commercial supplier, or laboratory-maintained line) and how long (estimated number of generation) they have been reared in your lab. Describe the rearing environment, such as temperature, humidity, and light/dark cycle (e.g. photoperiod). Specify the type and size of the rearing enclosures used (e.g. cages or crates). Detail the composition of the diet provided (e.g. nutritional content). Outline how the insects were allowed to reproduce (e.g. oviposition time, mating setup, oviposition conditions).

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