BRILL
WAGENINGEN
ACADEMIC

JOURNAL OF INSECTS AS FOOD AND FEED 11 (2025) S317–S339

Journal of Insects as Food and Feed
brill.com/jiff

RESEARCH ARTICLE

BugBook: Considerations for designing and performing insect larvae production experiments

David Deruytter[1]*, *Carl Coudron*[1], *Viktoria Wiklicky*[2], *Somaya Naser El Deen*[3], *Meggie Van Peer*[4], *Christos Rumbos*[5], *Daniela A. Peguero*[6], *Christina Adamaki-Sotiraki*[7], *Christos Athanassiou*[7], *Teun Veldkamp*[3], *Christoph Sandrock*[8] *and Wael Yakti*[9]

[1]Insect Research Centre, Inagro, Ieperseweg 87, Rumbeke-Beitem, Belgium; [2]Department of Energy and Technology, Swedish University of Agricultural Sciences, 756 51 Uppsala, Sweden; [3]Wageningen Livestock Research, De Elst 1, 6700 AH Wageningen, The Netherlands; [4]Centre of Expertise Sustainable Biomass and Chemistry, Thomas More University of Applied Sciences, Kleinhoefstraat 4, 2440 Geel, Belgium; [5]School of Agricultural Sciences, Department of Agriculture, University of Patras, 30200, Mesolongi, Greece; [6]Department Sanitation, Water and Solid Waste for Development (Sandec), Eawag: Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, 8600 Dubendorf, Switzerland; [7]Laboratory of Entomology and Agricultural Zoology, University of Thessaly, Phytokou Str., 38446, Volos, Greece; [8]Department of Livestock Sciences, Research Institute of Organic Agriculture (FiBL), Ackerstrasse 113, 5070 Frick, Switzerland; [9]Urban Plant Ecophysiology Division, Thaer-Institute of Agricultural and Horticultural Sciences, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Lentzeallee 55, 14195 Berlin, Germany; *david.deruytter@inagro.be

Received 1 December 2024 | Accepted 5 June 2025 | Published online 24 June 2025 | Published in issue 21 November 2025

Abstract

This article of the BugBook provides guidance and addresses challenges towards conducting experiments that aim to optimize the production of insects for food and feed, focusing exclusively on *Tenebrio molitor* (yellow mealworm) and *Hermetia illucens* (black soldier fly). The goal is to improve the reliability, reproducibility, and efficiency of insect production experiments. This reduces the human and financial resources needed, improves quality, and ensure the broader implementation of the results. Importantly, these guidelines are based on both data from literature and from unpublished experiences of the authors. Most aspects of the experimental systems are discussed, starting with methods to ensure that the laboratory conditions correct, followed by the experimental design and setup, including a focus on the control group, scale, and number of replicates. Thereafter, information is provided on the different aspects that need to be monitored during the experiment and how to conclude or harvest an experiment. The latter includes a discussion on the various criteria for determining the optimal harvest time. Finally, common challenges in working on insect larvae are identified and guidelines towards relevant publication information are provided.

Keywords

Hermetia illucens – insect production experiments – reproducibility – standardisation – *Tenebrio molitor*

Published with license by Koninklijke Brill BV | DOI: 10.1163/23524588-BJA10252
 | ISSN: 2352-4588 (online)

1 Introduction

The improvement and optimisation of products derived from more conventional livestock, including warm blooded animal husbandry, aquaculture, and apiculture has long been central to modern agriculture. These advancements have resulted in significant gains in the yield of milk, meat, and honey, amongst other products. However, compared to conventional farming, the production of insects for food and feed is a young industry that continues to face challenges related to efficiency optimisation and scalability. In recent years, there has been a considerable development leading to advances in scientific knowledge and industrial growth (Megido *et al.*, 2024). The majority of recent publications focuses on two insect species: *Tenebrio molitor* (yellow mealworm, MW, e.g. Kotsou *et al.*, 2023, 2024; Ribeiro *et al.*, 2018) and *Hermetia illucens* (black soldier fly, BSF, Athanassiou *et al.*, 2024).

This BugBook article aims to guide researchers in conducting experiments to improve the production of insect larvae. It provides a brief overview of the potential variables, advice on the laboratory and control conditions, experimental design, running, and ending a production experiment, before listing some of the frequent pitfalls. Finally, recommendations for publication are provided. It is important to note that these guidelines are based on both data from the literature and from unpublished experiences of the authors. This article will focus exclusively on BSF and MW larvae, but most of the recommendations may have conceptual merits for other farmed insect species.

It is important to consider that there are other methods that can be employed to improve production, which are detailed in other articles of the BugBook, such as genetic selection (Sandrock *et al.*, 2025), microbiome modification (Auger *et al.*, 2025), and improving reproduction (Tomberlin *et al.*, 2025). Detailed information on how to maintain a BSF or MW population is provided in Coudron *et al.* (2025).

2 Production variables

Insect production can be influenced by a multitude of variables ranging from broad, farm scale operational procedures, to the climate, the housing of the insects and finally the substrate they consume. Below some examples are presented to give an indication of the sheer abundance of, and interactions between, parameters that impact the production.

Operational procedures

The scale at which insects are produced (e.g. lab vs farm scale) and associated operational procedures can influence the production. The availability and experience of staff (students), workspace, equipment and financial possibilities are factors that will put practical and physical limits on the experimental design. This, for example, will determine the number of replicates or the number of larvae available for experimentation and will in turn determine the statistical power (see also Smetana *et al.*, 2025) of the experiment and the possibility for implementation by the industry (lab scale vs farm scale). Irrespective of the resources, there are other decisions that are made on a farm level that can impact the performance. Two examples illustrate this point. Firstly, different feeding regimes, such as single batch versus multiple batch feedings, can impact larval development (Dzepe *et al.*, 2021; Meneguz *et al.*, 2018). Secondly, in most instances, insects are redistributed at least once. This is done either to reduce the density or to have a fixed start of the experiment (see Designing an experiment). Yet, the timing will influence further growth. For example, starting an experiment using larvae with a fresh larval weight of 4 or 20 mg larvae (which may correspond to five- and seven-day old BSF larvae) can influence both survival and growth on an experimental diet. Finally, the act of monitoring larval growth itself may both improve the outcome as problems are detected early but may unintentionally also alter the outcome due to disturbance (light, handling, sampling).

Climate

In many cases, the local climate will not be optimal or even sufficient to grow BSF or MW larvae and therefore they are typically reared in climatized rooms. Several studies have already reported on the optimal climate conditions for BSF and MW larvae, with publications in BSF rearing reporting ambient environmental conditions around 27-30 °C and 50-70% relative humidity (RH), (Chia *et al.*, 2018; Holmes *et al.*, 2012; Shumo *et al.*, 2019; Wang *et al.*, 2023) while MW require around 27 °C and 60-80% RH (Deruytter *et al.*, 2023a; Eberle *et al.*, 2022; Johnsen *et al.*, 2021; Parsa *et al.*, 2023). However, several knowledge gaps remain that require further investigation. For example, regarding the specific environmental needs of the different larval instars as well as between larvae and adults (Lemke *et al.*, 2023). In addition to research on average climate conditions, the impact of the climate variability, such as day/night fluctuations, should be further investigated. In academia, near-complete climate control is often desired, but this

is not the case in most insect farms as tight control is expensive. Furthermore, preliminary evidence suggests there might be more flexibility than assumed. For example, Coudron *et al.* (2022b) observed only small changes in MW larval growth between a greenhouse and a tightly regulated climate room.

Finally, while "climate conditions" typically refer to ambient temperature and RH, further research is needed on ventilation and air quality. Ventilation can change the moisture evaporation and heat transfer from the substrate resulting in drying and cooling of the substrate. If the ventilation is not uniform, this can also increase the variability of the study. Ventilation will also alter the air quality by changing the air exchange rate. Research on emissions of insects during rearing (e.g. carbon dioxide and ammonia) is still limited (Boakye-Yiadom *et al.*, 2022; Mertenat *et al.*, 2019; Parodi *et al.*, 2021; Rossi *et al.*, 2024). More importantly, to our knowledge, no data exists on the maximum concentrations of different gasses before adverse effects are observed on the insects. As a point of reference, we suggest using the guidelines or rules that apply for livestock or human working environments in your country.

Housing

Within the climate room, insect larvae are typically reared in plastic crates at a specific larval density. Yet, the density of the larvae used in the experiment has a profound effect on the outcome of the experiment (Barragan-Fonseca *et al.*, 2018; Niu *et al.*, 2022; Parra Paz *et al.*, 2015; Yakti *et al.*, 2022). It is therefore important to consider this carefully in every experiment. Currently studies typically use larval density as the number of larvae per given surface area of the crate or container (e.g. cm^2). However, the larvae can move vertically through their substrate and are therefore not bound to a two-dimensional surface. It may be beneficial to express larval density as the number of larvae per given volume of feed (e.g. cm^3). For example, the available volume, not the surface area will determine the growth of MW larvae in wheat bran (Deruytter *et al.*, 2022). The actual density that larvae experience can vary significantly. This can be due to the natural tendency to aggregate (particularly BSF) or incorrect management practices such as the uneven distribution of wet feed for MW (Deruytter *et al.*, 2021). As a result, larval growth may become more variable.

Besides density, several studies have already indicated that the size and shape of the crate is important (Biasato *et al.*, 2024; Yakti *et al.*, 2022), but this variable is not yet fully understood. For example, ventilation effectiveness might be influenced by the crate design which determines the space between the crates. This in turn will alter, as mentioned before, evaporation and heat dissipation through ventilation. Although the authors acknowledge that other housing units than rectangular crates have been assessed and potentially used (Ingram 2018; Ko *et al.*, 2021; Peng *et al.*, 2022), this article will focus on the use of plastic rectangular crates, of different sizes, as the current standard in research.

Substrate

In each crate, feed is provided for the larvae. For BSF and MW larvae, the feed not only serves as their source of nutrients, but also as their living environment. Therefore, the term "substrate" will be further used instead of feed, to cover both aspects. The physical, chemical, and biological properties of the substrate play a crucial role in larval development. A few examples, research shows that particle size (Naser El Deen *et al.*, 2022; Peguero *et al.*, 2024; Yakti *et al.*, 2023), bulk density (Yakti *et al.*, 2023), water content (Bekker *et al.*, 2021; Chang *et al.*, 2017; Frooninckx *et al.*, 2024), pH (Coudron *et al.*, 2022a; Meneguz *et al.*, 2018), and the microbial community of the substrate (Bruno *et al.*, 2019; Wynants *et al.*, 2019) can impact the production dynamics and outcomes.

Due to the importance, assessing substrates is one of the most investigated factors to improve larvae production (Athanassiou *et al.*, 2024; Ewusie *et al.*, 2018; Gold *et al.*, 2018). However, the experimental methods reported in published literature differ considerably in design, scale, calculations, among other factors. Bosch *et al.* (2020) and Deruytter *et al.* (2023b) provided some specific suggestions for standardization of BSF experiments to increase harmonization amongst research studies. Similar work is published for MW experiments (Deruytter *et al.*, 2025). There are several innovative methods published with specific goals. For example, Gold *et al.* (2020) published a method to produce sterile larvae and substrate. This can help future research indicating the influence of the microbiome on the substrate conversion capacity and nutrient requirements of the larvae. Morales-Ramos *et al.* (2020) introduced a method for MW to self-select substrate ingredients, potentially increasing the speed and efficiency for substrate selection and combinations. Interestingly, altering the larval density can be used to estimate the apparent digestibility; a parameter that is frequently difficult to determine (Guillaume *et al.*, 2023).

Finally, it is increasingly evident that not only the ambient room climate conditions, as described before, but also substrate 'environmental conditions' (e.g. tem-

perature) play a crucial role. This is particularly important for BSF larvae as the substrate temperature can exceed 40 °C (Li *et al.*, 2023). While this also applies MW larvae, the effect is less pronounced (Deruytter *et al.*, 2022). Controlling the temperature of the substrate is challenging, as it is influenced by numerous variables such as, moisture content of the substrate (Cheng *et al.*, 2017), larval density (Yakti *et al.*, 2022; Deruytter *et al.*, 2022), insulation capacity (substrate height, composition and density) (Biasato *et al.*, 2024), and air circulation (Palma *et al.*, 2018).

3 Larvae production experiments

Assessment of laboratory conditions

Experimental set-ups (e.g. scale) and laboratory conditions (e.g. temperature stability of the climate room) vary greatly between institutions and may influence the repeatability and reproducibility of an experiment. Therefore it is highly recommended to assess both the experimental equipment (e.g. heating and ventilation of the climatised room) and methodology (e.g. subsampling protocol) combined with adequate training for staff. Firstly, the equipment should be assessed, ensuring it works well. For example, for a climate room this means that the ambient climate should be homogeneous throughout the room (e.g. minimize differences between floor and ceiling, especially when working with stacked crates) and the sensors are calibrated. Secondly, a validation process can be initiated. For the initial validation process, a triplicate of a standardised, well known, and stable substrate should be used (e.g. wheat bran). The collected data can then be compared to estimated literature values. For example, studies by Deruytter *et al.* (2023b) for BSF and Deruytter *et al.* (2025) for MW provide both averages and expected variability in larvae size and overall yield based on a standardised protocol, but other literature can be used depending on the set-up. If these results deviate from the literature values (e.g. higher than expected mortality), an adjustment of the laboratory conditions or further training of staff or students may be required to improve the process. Data collection should begin once the collected numbers fall within the target range. Validating these numbers should be standard laboratory routine as regularly monitoring larval growth and survival can also serve as a historical control. Over time, this data helps to quickly identify deviations from the standard in the assessed set-up. For example, a real-world case, an experimental control resulted in BSF larvae with fresh weights of 65 mg and 70% survival, yet the in-house historical data indicated that the control should have an average fresh weight of 100 mg and 90% survival. This was a clear red flag, casting doubt on the reliability of the results prompting a thorough reassessment of the conditions. In this case, the discrepancy in the results was caused by a malfunctioning sensor but a myriad of other factors could potentially cause deviations of the norm. Issues can be caused by a malfunctioning ventilation system resulting in an inadequate climate, over or underfeeding of larva by initial calculation mistakes, mistakes during initial larval dosage, inbreeding depression, etc.

Designing an experiment

Constructing a randomized experiment (a setup where experimental units are randomly assigned to different treatments) starts by defining the hypotheses, including both the null and alternative hypothesis. Next, an adequate experimental design should be determined to test the hypotheses with relevant response (or dependant) variables, a robust protocol, and finally a correct statistical analysis of the data. For the latter see Smetana *et al.* (2025). Controlled experiments aim to isolate variables and test specific hypotheses under controlled conditions. This can be done by manipulating one or more variables (independent variable(s), e.g. substrate) while observing the differences between treatments in a measured parameter (the dependent variable, e.g. larval growth). A control group would serve as a baseline and be compared with the experimental group(s), in which the conditions are manipulated. In order to set up an experimental procedure accurately, several parameters should be carefully considered such as the number of individual larvae used in each experimental unit (i.e. larval density), the initial fresh weight of individuals, the amount of substrate provided, as well as substrate (pre)treatment. Depending on the insect species and the hypotheses tested, the aforementioned procedures may be addressed differently.

Although the whole experimental setup must be critically designed to obtain high quality and representative data. There are three consideration that require a more in-depth discussion: the control, the scale, and replicates that are important for all species and life stages. Thereafter more specific information is provided on the experimental design using BSF and MW larvae.

Experimental control:

The importance of the experimental control cannot be overstated. A well-designed control group serves as a baseline to evaluate the effects of treatments while

minimizing the influence of confounding variables. This enables the determination whether the changes in the response variable (e.g. a larval weight) are directly attributed to the independent variable (e.g. substrate). The control group also validates experimental results and provides a reference point for future studies, both within the same research group and across different laboratories. This is crucial for ensuring that findings can be replicated under similar conditions. For example, in a feeding experiment, if larvae in the treatment group show reduced growth, it may be assumed that the substrate lacks essential nutrients. However, if the larvae in the control group also show signs of unexpected growth reduction the cause may not be exclusively attributed to the substrate, as other factors such as insecticide contamination might have influenced the growth. To ensure a valid comparison, the treatment and control group conditions must be identical except for the experimental factor(s) being tested. This means using larvae from the same production batch for both the control and treatment group to ensure a common parental genetic background and uniform nursery conditions. Unless you want to assess the influence of the genetic background or nursery conditions. Furthermore, all treatments of the experiment should be conducted under the same experimental conditions including larvae density, substrate properties, -load, -depth and -regime, abiotic exposure (i.e. temperature and RH), same batch of larvae.

When conducting experiments to test larval performance on different substrates, special attention should be given to the control substrate as it presents specific challenges. A control should be established using a reference diet (e.g. chicken feed, Gainesville diet or wheat bran), detailing the proportions of ingredients and the commercial supplier, and specifying any modifications made (e.g. sieving and grinding; Bosch *et al.*, 2020). Currently, there is a lack of standardized substrates available on a global scale. Many BSF studies report chicken feed as a standard control substrate, this presents a major challenge, as "chicken feed" is not a uniform global blend but rather varies significantly in terms of nutritional value and characteristics. This is also partly true for wheat bran, which is the standard control substrate used in production experiments with MW, and the Gainesville diet. Although less variable than "chicken feed", the nutritional composition and physical properties (e.g. particle size) of wheat bran still varies among batches and suppliers, affecting MW larvae performance (Deruytter *et al.*, 2025). The evaluation of a standard protocol for MW through a ring-test with several international partners recently revealed modest variability among partners attributed to the different wheat brans used as control substrate (Deruytter *et al.*, 2025). Differences in grain or particle size, pellet size or form, and the nutritional components, can lead to differences in the performance of the larvae. Such metadata, especially the macronutrient composition, should be reported to allow proper comparison. A fully artificial diet based on products with a stable composition is, to our knowledge, not yet available.

Depending on the hypothesis, the complexity may increase as the experiment could include a positive control, a negative control, or both. A positive control refers to the group treated under conditions known to yield a positive outcome (Gross *et al.*, 1967). In the case of production experiments, a positive control treatment can be larvae growing on a high-quality substrate when testing different substrates, or larvae exposed to optimal growth conditions when assessing the influence of the abiotic condition on larvae performance. A positiive control, besides serving as the reference to quantify the effect size (strength or magnitude of the difference between treatments), validates that the other conditions of the experiments are suitable for the larvae (e.g. the ambient temperature at which different substrates are tested is within the optimal, or at least acceptable range). On the other hand, a negative control is introduced to demonstrate that any observed positive effect is attributed to the treatment and not to other confounding variables that created background noise or false positive effects (Penning de Vries and Groenwold, 2023; Yakti, *et al.*, 2025). However, the necessity of including negative, positive, or both controls should be assessed and judged case-by-case depending on the research question. Despite providing a more comprehensive understanding of the phenomenon being studied, including both negative and positive controls increases the number of treatments, and the efforts required to conduct the experiment.

Choosing the right type of control will save time and effort, and in some cases a positive control might not be required. For example, a negative control is necessary when the aim is to test the effect of a substrate additive, or the effect of pretreating the substrate on larval performance. The hypothesis can be that the additive would confer superior performance to the larvae receiving this substrate, and the group receiving the additive would be compared with a group that received a substrate without the additive or the pretreatment, serving as the negative control (Peguero *et al.*, 2023). For example, additive bacterial strains and fermentation processes have

been used to enhance BSF rearing substrate, with the performance of the larvae assessed on the treated substrates and compared with the performance of larvae on a negative control (untreated) substrate (Gorrens *et al.*, 2023; Meng *et al.*, 2023; Witriana *et al.*, 2023).

Experimental scale:
Understanding the different larval performances across different scales is essential for both researchers and commercial producers. Industrial insect production is usually conducted at large scales (e.g. in 60 × 40 cm crates or larger) while most published research report on experiments with smaller crates. It can be challenging and sometimes incorrect to extrapolate this data due to differences in process parameters. For example, documented differences in substrate temperature development and evaporation among scales can alter BSF larvae performance. On the one hand, initial testing can be conducted on a small scale, providing a cost-effective approach to screen a wide variety of dietary treatments. This approach yields valuable insights and allows for high statistical power through allowing time and cost-efficient replications. Laboratory bioassays have been proven effective in screening a wide range of substrates for both BSF (Bellezza Oddon *et al.*, 2022; Resconi *et al.*, 2024) and MW larvae (Oonincx *et al.*, 2015; Rumbos *et al.*, 2021). For example, small-scale experiments testing factors such as nutritional values of substrates would likely result in similar trends across the scales. When larvae thrive more on a substrate with higher nutritional value compared to other substrates, the substrate with the high nutritional value is likely also superior if the experiment was upscaled, but the absolute production parameters (e.g. larval biomass produced per substrate weight, production duration, etc.) would likely vary depending on the scale. Large-scale trials, on the other hand, better simulate industrial production conditions and generate results easily adopted by the industry. Several large-scale experiments have been published for both BSF (Scala *et al.*, 2020; Schøn *et al.*, 2025) and MW larvae (Deruytter *et al.*, 2021; Vrontaki *et al.*, 2024). However, the different experimental scales in published research is among many aspects that often complicate direct comparison of results among studies. Therefore, standardised experimental protocols have been recently suggested for experiments with BSF (Deruytter *et al.*, 2023b), as well as MW larvae (Deruytter *et al.*, 2025). The suggested use of a unified crate size (60 × 40 cm) could theoretically eliminate scale-caused inter-study variance.

A critical factor to achieve efficiency in translating small-scale results to large-scale production is deep understanding of how the tested parameters (dependant variables) differ among scales. Scale effects usually occur in industrial bioprocesses even in well-controlled and insulated bioreactors, and when using other biological systems. Various papers have already reported the influence of scale on the performance of BSF larvae and MW. It is generally shown that on a large scale, BSF Larvae achieve similar or better performance, and higher bioconversion efficiency (Biasato *et al.*, 2024; Schøn *et al.*, 2025; Yakti, 2022; Yang *et al.*, 2020), while in MW the performance of larvae does not seem to improve in a bigger scale (Adamaki-Sotiraki *et al.*, 2024)

A known example, in the case of BSF, is that changing the scale of rearing can alter the thermodynamics of the production process. As observed by Yakti *et al.* (2022), bigger crates (2060 cm^2) compared to smaller crates (194 cm^2) had higher substrate temperature which could be, among other reasons, due to the higher heat capacity. Heat capacity is known to be proportional to the total amount of a substrate, which means that larger amounts of substrate are less affected by ambient temperatures (Bergman, 2011). In contrast, smaller scales are more likely to exhibit a higher rate of proportional energy loss. Additionally, different crate dimensions manipulate the surface area of the substrate; the surface area-to-volume ratio influences heat transfer and evaporation, ultimately influencing the larval performance. These physical effects can be more pronounced in the case of BSF due to the moisture content of the substrate, which magnifies the influence of thermal capacity.

Replicates:
Replication enhances the reliability and validity of results by promoting precision in the obtained data. Special care should be taken when conducting production experiments with farmed insects to base the findings on true experimental replicates, instead of pseudo-replicates. Initially described by Hurlbert (1984), pseudo-replication occurs when observations and measurements are not statistically independent. In practice, it means that measurements should not be taken on individuals, but on the experimental units. In other words, the individual MW or BSF larvae of the same crate are pseudo-replicates, and weighing 50 larvae from the same crate does not equal 50 replicates. The average of the 50 measurements estimates the average larval weight of that replicate.

The number of required replicates depends on several factors. Firstly, the expected change in the dependent variable, a 50% growth difference is easy to spot compared to 5%. Secondly, on the variability of the variable in question. Some initial information on the variability can be extracted from Deruytter *et al.* (2023b) for BSF or Deruytter *et al.* (2025) for MW. As an example, in those studies it was clear that the variability in average weight from smaller larvae is greater than at harvest and that BSF experience a greater variation than MW. The minimum number of replicates can be estimated using an *a priori* power analysis when you have knowledge on expected effect size and population variability (e.g. Charan and Kantharia, 2013). Furthermore, it is expected that in the near future, the *a priori* power analysis will become more important with increasing animal welfare standards. If no reliable information or estimation is available or possible, the rule of thumb for most animal studies is to use six replicates (n = 6) for every treatment when comparing two groups (a total of 12 experimental units). More information is provided in Smetana *et al.* (2025).

Mealworm larvae experiments:
For the MW, experimental procedures need to be adjusted depending on the scale of the experiment, whether it is laboratory-based or conducted on a larger scale. The appropriate larval density is a crucial factor in achieving reliable and reproducible results (Deruytter and Coudron, 2022; Weaver and McFarlane, 1990). Besides a similar density, a uniformity in larval size is also recommended. A uniform larval size can be achieved via sieving. For example, two sieves with openings of 850 μm and 600 μm, could be used to collect larvae of similar head capsule size. Larvae passing through the 850 μm but retained by the 600 μm sieve can be effectively selected. Notably, the study conducted by Morales-Ramos *et al.* (2015) includes a comprehensive table that correlates head capsule size with larval age.

In laboratory-scale experiments, larvae that are 14 days old are typically used. Groups of 50 individuals can be inserted in an experimental unit (e.g. plastic cylindrical vials 7.5 cm in diameter and 8.8 cm in height (Gulsunoglu-Konuskan and Dag, 2024). Prior to the insertion of larvae to experimental units, proper substrate preparation and distribution are essential for maintaining experimental accuracy. For non-powdered substrate, grinding is recommended to facilitate optimal consumption by the larvae (Naser el Deen, 2022). The amount of substrate provided should also correspond to the density of larvae. For example, 50 larvae consume approximately 10 g of control substrate, such as wheat bran. In addition to dry substrate, MW larvae require a source of water along the whole experimental procedure to reach their optimal growth. Agar cubes, cut into 1 cm^3 pieces and prepared with a concentration of around 20 g/l, are recommended as a standardized moist component. Care must be taken to provide enough agar. Regarding the frequency and the quantity of agar provision, usually when a certain parameter is tested, MW larvae would differ in their growth and so does their agar consumption. Remove any remaining agar upon the provision of fresh agar to avoid the growth of, potentially harmful, micro-organisms.

For large-scale experiments, e.g. 10 000 larvae in a 60 × 40 cm^2 crate (Deruytter *et al.*, 2025) 4-week-old larvae are preferred. MW larval density generally ranges between 0.6 and 10.4 larvae/cm^2 depending on the substrate hight. On a volume basis, a density of 1 larvae/cm^3 on wheat bran is a good rule of thumb (Deruytter and Coudron, 2022, Deruytter *et al.*, 2022). The proper substrate preparation and distribution are essential for large-scale experiments as well. An amount of 10 000 larvae requires about 2,000 g of wheat bran. Same as laboratory-scale experiments, the provision of moisture is also essential for large-scale experiments, but distribution of the agar becomes much more important. To ensure a good and equal growth, the agar cubes should not be more than 10 cm apart (Deruytter *et al.*, 2021).

For both laboratory and large-scale experiments, it is important to note that substrate consumption may vary depending on the type of by-products or diet formulations used. Depending on the goal of each experiment, it is recommended to avoid the provision of the substrate ad libitum. Apart of economic aspects and undermining key sustainability arguments, there are strong indications that, at least some insects can, and will, choose what they eat and if provided ad libitum they may choose only a, non-representative, part of the provided diet (Morales-Ramos *et al.*, 2020). Furthermore, it is commonly known in animal nutrition science that accurate dietary requirements and feed conversion rate (FCR) estimates are best generated when feed is limited (Heuel *et al.*, 2022). Experiments can, therefore, either start with a pretrial to assess the substrate consumption, which is preferred, or begin with a standard amount of substrate and additional substrate is provided as needed. The latter can be particularly challenging to determine when it is 'needed' and should be described in any publication. All supplemental substrate added during the experiment must be recorded to ensure consistency across treatments.

Black soldier fly larvae experiments:

In the case of BSF larvae experiments, many aspects of the experimental design are similar to mealworms. As a start, the larvae used should derive from the same batch with maximum uniformity in their age and weight. To reduce the age variance within the batch it is recommended to start with neonates that hatched within 24 hours as described by Deruytter *et al.* (2023), these are sometimes called 0-DOL (0-day-old larvae). The neonates are usually grown on a control substrate for 5-7 days (e.g. Gainesville diet or chicken feed) until reaching a mean fresh larval weight and size that enables their handling and counting (generally between 2 and 10 mg fresh weight/larvae). In any study, it is necessary to provide both the age and initial average weight of the larvae used in the experiment, preferably fresh and dry weight. Usually, the crates are filled with wet substrate, and the larvae are put on top after quantification. A good experimental setup should have a suitable larval density to enable full substrate consumption and the development of larvae to the non-feeding pre-pupae stage. Large-scale set-ups typically aim to add around 10-20 000 larvae per experimental crate (60 × 40 cm) that can be filled with 10 kg of wet substrate. This corresponds with larval densities between 5-10 cm^2. These values can be downscaled to adjust the experimental setup, and the differences between scales may be minimized with proper climate control (Schøn *et al.*, 2025). The substrate can be applied as a single batch or in multiple times. Batch feeding is less time consuming while splitting the feeding over multiple times allows for adjustments and allows substrate consumption before a new substrate is added, leading to increased aeration and better substrate temperature control. Single batch feeding is, however, commonly adopted in industrial settings. Substrate consumption, however, can vary based on the nutritional content and it is always advisable to run a pre-test to adjust the amount of substrate given to each larva in the experimental system. Furthermore, substrates that differ in their composition and nutritional value will highly likely differ in their physical properties as well (e.g. particle size and viscosity). These physical properties can influence larval performance (Fuhrmann *et al.*, 2024; Peguero *et al.*, 2024; Yakti *et al.*, 2023) and should be considered when interpreting the results. A major difference between BSF and MW is the huge importance of the moisture content of the substrate. When formulating the substrates, establishing an optimal moisture content is not straightforward. A rule of thumb of keeping the moisture content close to 70% is commonly used in scientific research. However, the optimal moisture content depends on different physical characteristics of the substrate. Water holding capacity, for example, is specific to each feeding substrate (Ramanzin *et al.*, 1994; Yakti *et al.*, 2023). Furthermore, substrate degradation (e.g. when vegetables and fruits are used as feeding substrate) can lead to water release resulting in a free water phase a few days after starting the experiment, which ultimately increases larvae mortality (Bekker *et al.*, 2021; Peguero *et al.*, 2024). It is essential to thoroughly homogenize the diet to ensure uniformity in substrate composition and distribution across the unit, and it is important that no free water is observed. The substrate preparation and homogenization prior to portioning are key steps in keeping consistency across all experimental units, minimizing variability in the outcomes. Especially in large scale tests, the moisture content of the substrate can decrease during the experiment as the evaporation is accelerated further by the heat generated by the larvae. In some cases, this drying can go so fast that the larvae cannot consume the nutrients in the feed in time. Adding water to the substrate during the experiment could solve the issue. This must be based on a specific and reasonable criterion (e.g. keeping the weight of the crate at a certain level) and must be mentioned in the material and methods of any paper. Given the differences in substrate physics and thermodynamics based on the rearing scale, the need for additional water will differ and must be assessed case by case.

Conducting the experiment

After formulating the hypothesis, designing, and initiating the experiment, the experimental conditions must be closely monitored to minimise unwanted variations. Monitoring abiotic conditions, such as ambient temperature and RH, is particularly important as these may fluctuate throughout the course of the experiment. Fluctuations may occur due to technical errors (e.g. electrical malfunctions), human errors (forgetting to close a door or blocking the ventilation) or changes in the weather (e.g. rapid shifts in outside temperature). Ideally, these parameters are monitored continuously but they should be measured at least daily. The average and standard deviation of the ambient temperature and RH should be stated in a publication together with any adjustments, changes, or aberrations during the experiment. Besides the ambient temperature and RH, it is advisable to measure the concentration of CO_2 and ammonia throughout the experiment for the safety of personnel. To mitigate exposure, adequate ventilation and staff training should be considered in the plan-

ning. Finally, it is highly recommended to also track the temperature conditions within the crates. As mentioned before, in large-scale experiments with high densities (e.g. >5 larvae/cm^2), the substrate temperatures can be significantly higher than the ambient temperature (Bloukounon-Goubalan *et al.*, 2020; Deruytter *et al.*, 2022; Gold *et al.*, 2020; Yakti *et al.*, 2022). Furthermore, the temperature change highly depends on the external environment such as the ventilation rate, ambient temperature, and RH (Lalander *et al.*, 2020; Padmanabha *et al.*, 2023) and substrate properties such as porosity, initial moisture content, and the microbial load (Abduh *et al.*, 2022; Agnew and Leonard, 2003; Hansen *et al.*, 2004; Lalander *et al.*, 2020; McEachern, 2018; Schreven *et al.*, 2022).; Yakti *et al.*, 2022Therefore, tracking the substrate temperature throughout the experiment can provide valuable information for the interpretation of the results. As an example, when substrate temperatures of more than 50 °C are reached within a BSF crate, potentially indicating a high microbial activity, this may result in suboptimal growth as the larvae may move away from the heated areas, limiting their access to the substrate and leading to an erroneous conclusions. Substrate temperature can be measured using remote sensors or via frequent direct probing using a thermometer (for example, see Peguero *et al.*, 2024). Infra-red-thermometers or cameras can also be useful tools to quickly assess substrate temperature and spatial variability. This may identify hot-spots as a result of the metabolic heat produced in combination with a heterogeneous distribution of the larvae (Li *et al.*, 2023; Shishkov *et al.*, 2019). Do keep in mind that these infra-red thermometers do only measure the surface temperature which may be substantially colder than the core. In addition to the substrate temperature, monitoring the pH of the substrate of BSF experiments is useful to interpret the results. In BSF rearing, the pH typically increases throughout the experiment (Meneguz *et al.*, 2018), tracking the change of pH can offer useful insights into the experiment as it may provide information on anaerobic (e.g. fermentation) and aerobic conditions (Coudron *et al.*, 2024).

Besides monitoring the abiotic conditions, it is important to monitor the larvae itself. While many experimental parameters can only be measured after terminating the experiment, three key biological parameters: average larvae weight, survival, and total yield can be determined. To determine the average single-larva weight the individuals should first be separated from the substrate. Thereafter, the average can be determined by either weighing individuals, or based on group-weighing where total weights are divided by the number of included individuals. The latter is more convenient, but the former gives an indication on the within replicate variation. Do keep in mind that, as described above, the individual measurements are technical replicates not true replicates. An alternative to weighing the larvae is to estimate the weight based on the length and width of the larvae (e.g. using microscope photos), this may be especially useful for small larvae (Ewusie *et al.*, 2019; Laursen *et al.*, 2021) or future AI based measuring systems (Nawoya *et al.*, 2024). These intermediate measurements can be used to construct growth curves (Smetana *et al.*, 2025) or help deciding when to terminate the experiment (see Harvest). The survival rate can be estimated directly by counting the larvae or is estimated by taking appropriate subsamples (large scale, see Larvae sampling) and extrapolating the number of larvae in the sample to the whole crate. Besides growth and survival, other parameters could be calculated such as the total yield at that given time point (the estimated average larval weight multiplied by their estimated number of larvae in the crate). We propose that for non-destructive sampling the larvae are placed back into the crate. This is especially the case in smaller set-ups where all or most of the larvae are disturbed (e.g. a 100 larvae sample in a 1000 larvae crate). For destructive sampling (e.g. for intermediate microbiome analysis; Wynants *et al.*, 2019), in combination with lab scale experiments, it is best to set-up additional replicates that can be removed upon sampling. The strong reduction in larval density would otherwise affect the remainder of the test. It is important to keep in mind that accurate random sampling may be difficult e.g. due to variance in larvae size and distribution. There is also a trade-off between sampling accuracy and the disturbance of substrate in the crate (see also Larvae sampling).

Next to the experimental parameters, there are several other aspects that need monitoring. A potential issue during the rearing phase of BSF is the escape of larvae triggered by excess moisture or temperature and made possible by water adhesion of larvae to crate walls (especially when RH is high and ventilation low). This causes a decrease in larval density, altering the overall process efficiency and an increase in individual larval growth rate (due to changes in feed amounts per larvae), and cause a bias in the survival estimates. Increasing the height of the crates and ventilation speed can reduce this as well as reducing the RH or initial substrate moisture content. If none of those methods are practical to implement, using a tightly closing lid with a screen to allow some air exchange is also possible. The latter is frequently used in small scale laboratory experiments. In

addition to escaping larvae, the presence of pests such as rodents or other insectivores can unintentionally lead to lower larval density. While this is less of a concern in controlled laboratory conditions, this may become a significant issue in a larger scale waste processing facility or an industrial setting if hygiene standards are not met. Finally, the provision of water (BSF) or wet feed source (MW) during the experiment is crucial to the outcome of the experiment as mentioned above and should be monitored closely.

Finally, an opportunity can be seized during monitoring or intermediate measurements to re-randomize the experiment. When the experiment has a randomized design the larvae and crates are assigned at random to different treatments at the start. The crates can be re-randomized during the experiment by placing the crates back in a random order in the rearing room. This will reduce the influence of differences in microclimate, which may otherwise lead to false conclusions.

Finish and harvest

Timing of harvest:

A crucial aspect of a production experiment is deciding when to end the experiment, further referred to as "harvest". The time of harvesting BSF and MW larvae varies depending on the experimental setup, the purpose of the experiment, and the parameters of interest. Treatments can all be harvested at one time point or at different time points. Most studies set a time point at which all treatments are harvested, which simplifies the experimental design and results interpretation by eliminating the confounding effect of time. The drawback of harvesting at a designated timepoint is that it could lead to substrate under- or over-consumption e.g. due to differences in substrate quality. This in turn would influence the biomass data and lead to bias in the feed conversion ratio calculation. Finally, it also depends on whether the research aims at solely assessing productivity, or also at investigating life history parameters in an evolutionary ecology context. Therefore, it is not meaningful to set a single criterion for harvesting as each option has benefits and drawbacks, but it is important to be aware of them and to report the method chosen.

The following criteria are commonly used as a basis for harvesting the larvae and the termination of experiments:

Fixed time for all treatments: Harvesting is done at a fixed predetermined time for all treatments regardless of larval development. This approach simplifies scheduling and consistency in experimental designs but may result in biased measurements of larval growth, development, and final yield, especially if growth rate differs between the treatments. The generated data related to substrate consumption and maximal larval growth (e.g. FCR and total yield) must be critically interpreted because when differences in larval growth are observed among treatments, it is less likely that the nutrients in all substrates are equally depleted. Furthermore, the biological age (e.g. instar) that affects larval composition independently of diets will likely vary between treatments and affect conclusions (Liu *et al.*, 2017). For example, some larvae may still grow while others already build up substantial fat reserves during later larval instars. Nevertheless, this criterion may relate best to production scenarios with fixed harvesting time that aim to maximize yield in the shortest production period.

First (pre)pupae appearance: This method suggests terminating the experiment upon appearance of the first pupae (MW) or prepupae (BSF). This approach has the drawback that the development of a single individual determines the timing for the entire experimental unit. For this reason, this method is not recommended.

Percentage of (pre)pupation: This approach suggests terminating the experiment when a percentage of larvae (i.e. 5-20%) have become (pre)pupae, providing a more generalized assessment of development time. However, in the last larval instar before pupation, larvae stop feeding, engage stronger in lipid catabolism, and lose weight accordingly. This may lead to an underestimation of the biomass. However, total body dry weight has been shown to remain stable across late 5th and 6th instars for BSF (Liu *et al.*, 2017), which suggests that this weight loss is solely due to empty guts and reduced water contents. Bosch *et al.* (2020) therefore proposed harvesting when 5% of the initial larvae are in the (pre)pupal stage, when most larvae are still actively feeding. However, estimating life stage percentages can be subjective, resulting in high standard deviations (Van Peer *et al.*, 2023). Furthermore, due to the uncertain nature of the (pre)pupation, planning a trial may be more difficult, especially for large trials which may require some lead time. Additionally, on some experimental diets pupal development might not be reached due to non-optimal substrate properties. For example, due to the presence of insecticides (Hill *et al.*, 2024). In such cases of stage-specific yield estimation, adequate FCR and related parameters can only be assessed realistically if additional feed rations are provided as needed (e.g. Sandrock *et al.*, 2022).

Growth monitoring: A more accurate, however labour-intensive, method that avoids the appearance of non-feeding stages while capturing peak growth is to monitor larval weight frequently. This can be daily for BSF larvae or every week for the slower growing MW larvae, especially near the end of their growth. The larvae of a particular treatment would be harvested when the mean larval weight of the treatment stagnates, ensuring larvae are harvested right after they have reached their maximum weight on the given substrate. In an experimental setup where the tested factors (independent variables) influence the performance of larvae, the point at which larval growth stagnates would differ among the treatments (Scala *et al.*, 2020; Yakti *et al.*, 2023; Yakti *et al.*, 2024). Therefore, the growth must be evaluated per treatment. The harvest parameters (e.g. FCR, total yield) will be more accurate, but the interpretation of the results should also consider that the time of harvest influences many other parameters related to larval physiology and possibly their nutrient composition. Besides being labour intensive this method is also intrusive and handling the crates frequently may alter the results.

Harvest:

The harvesting technique varies depending on the scale of the experiment. In laboratory-scale experiments, which typically involve 100-1,500 larvae per rearing crate, larvae can be picked from the substrate manually using forceps. However, for pilot or larger-scale experiments involving thousands to millions of larvae, harvesting is often achieved by separating the larvae from their substrate through sieving (manual or automatic vibrating screen). In practice, there are several challenges related to the harvest of these insects (Cheng *et al.*, 2017). The harvestability of insects through sieving is influenced by several factors, including:

(1) environmental climate conditions (ambient temperature, aeration, and RH)
(2) larval density
(3) substrate properties such as moisture content, water holding capacity, particle size, volume, layer thickness, porosity, cohesion, etc.
(4) larval performance on these substrates

Mealworm harvesting: Harvesting MW larvae is, in general, straightforward. The frass and any leftover substrate should be dry and sievable on a 2 mm sieve. Frass even passes through a 0.5-mm sieve. Only leftover substrate particles above 2 mm and the exuviae (the moulted exoskeletons when larvae change instar) may pose some additional work. For the latter using ventilation or vacuum can be useful as they are very light. Keep in mind that sieving mealworm crates can produce a lot of fine, airborne particles (e.g. frass) due to the low moisture content and it is possible to become allergic to mealworms. It is highly advised to at least wear a FFP3 mask during this operation.

Black soldier fly harvesting: Harvesting BSF can be more challenging. As mentioned above, BSF substrate has an initial moisture content of around 70% at the start of the experiment. In most cases, a moisture content of 70% is too high for direct dry sieving. Fortunately, during the experiment the substrate dries depending on a multitude of factors. For example, larval density and age significantly affects metabolic heat production and thus substrate temperature, which in turn enhances moisture evaporation from the substrate (Laksanawimol *et al.*, 2024; McEachern, 2018; Yakti *et al.*, 2023).

In reality, using non-optimized experimental diets, including many barely pretreated waste streams, can thus result in crates that are very difficult to harvest. Frequently, substrates are not fully processed due to their suboptimal composition, leaving larger frass aggregates, complicating the sieving process. Insufficiently dehydrated substrates at the time of harvest can become sticky, making sieving impossible as moisture levels may affect the frass's particle size, as water's adhesive properties cause particles to aggregate when moisture is high. As an example, although not in crates, Cheng *et al.* (2017) evaluated different food waste moisture contents on sieving efficiency at harvest. It was found that using food waste with 70 or 75% moisture content resulted in the frass's moisture content gradually dropping to about 50%, producing a fine frass that could easily be sieved. Conversely, food waste with 80% moisture content led to a frass that remained over 80% moisture that initially forms granules and eventually becoming a sticky slurry.

An alternative harvesting method can be used for frass that cannot be dry sieved. This involves separating BSF larvae from their substrate by washing or wet sieving. Using this approach, the substrate is washed away leaving only the clean larvae behind if the particles are small enough. In a next step, the larvae are dried using paper tissues (Bosch *et al.*, 2020; Coudron *et al.*, 2024). However, this method does make it difficult for the frass to be weighed directly and complicates sampling for chemical analyses. The total frass weight can be calculated from the total crate weight minus the empty crate weight and harvested larvae. Representative frass samples for chemical analysis should be taken before washing.

Performance parameters:
At the end of the experiment, a series of parameters or dependant variables can be measured to evaluate the performance. In addition to the more commonly reported parameters such as average larval weight, survival, and total yield (see 3.3), several other factors are recommended to measure in all conducted experiments. An overview is provided below and in Table 1.

Firstly, the dry weight content (% DW) of the larvae is an important parameter. While it is straightforward to measure and provides valuable insight, it is not always reported in studies. Differences in yield or average larval weight may be attributed to variations in multiplied with live larva yield, % DW provides the key metric for the insect industry: dry weight yield.

Feed conversion rate (FCR), bioconversion rate (BCR) and efficiency of conversion index (ECI) are amongst the most important metrics, though they are closely related. For comparability among studies and within a study, it is recommended to calculate and present these parameters on a dry weight basis (e.g. FCR as dry substrate/dry weight gained). Calculations are shown in Table 1, which require information on the initial and end larvae weight (wet weight and % DW), and the initial and end substrate weight (wet weight and % DW). As mentioned before, meaningful values can only be calculated when the larvae are not fed ad libitum. It is recommended to report at least one of these metrics (FCR, BCR or ECI), along with the corresponding formula. The latter is important as slightly different formulas may be used depending on the field of research. Providing the original data needed for these calculations enables other researchers to recalculate other parameters, which is particularly important for meta-analyses and is, therefore, highly encouraged. These metrics can be further refined to focus on specific nutrients in the substrate such as the nitrogen-FCR to estimate protein efficiency (e.g. Sandrock *et al.*, 2022). An underestimated factor is the influence of gut content, which may skew calculations. Compared to conventional livestock, removing gut contents to evaluate carcass yields is difficult to impossible. However, methods such as starvation could help emptying the gut but this cannot be ensured unless the gut content is manually removed after killing for each insect (Dortmans *et al.*, 2017; Wynants *et al.*, 2017). Finally, chemical and microbiological analysis of the larvae and frass can be done and are detailed in Smets *et al.* (2025) and Auger *et al.* (2025).

4 Challenges

In the following section some of the challenges of conducting production experiments are discussed. Because many of these are rarely reported in published literature, this part does rely to a larger extent on the combined experiences of the authors.

Larvae sampling

Sampling larvae is a standard practice in larger experiments and is used, for example, to determine both the average weight and number of individuals or survival. Moreover, accurately estimating the number of larvae at the beginning of the experiment is crucial as the initial larval number can influence the process parameters during the experiment. This is, for example, due to the negative correlation between the average weight of the individual larvae and the density in feed restricted situations. Given the importance, it is recommended to take the average of, at least, three technical replicates (three samples from the same crate) to estimate the number of larvae. We furthermore recommend calculating the coefficient of variation (CoV) on the technical replicates (standard deviation/mean of the technical replicates) and only use the estimated value if the CoV is below 0.1. Or in other words, when the standard deviation is less than 10% of the estimated mean.

Although it may seem straight forward, the method of sampling can have significant impact on the reliability of the result. First and foremost, the sample should be representative of the entire crate. A single scoop from the top layer of a large crate is unlikely to provide an accurate representation. Both experience and scientific literature report that larvae tend to distribute non-randomly, especially when disturbed (Li *et al.*, 2023; Shishkov *et al.*, 2019). Therefore, it is essential to thoroughly homogenize the contents of the crate with sample(s) taken during or immediately thereafter. In some cases, homogenization of the larvae and substrate may not be possible or desirable. Particularly in experiments where certain substrates or methods are negatively affected by disturbance, such as layering (e.g. wheat bran and frass layer for MW), aeration or bioturbation. Taking multiple samples to estimate the variability (e.g. four corners and centre) or add additional replicates for destructive sampling may overcome this issue.

Besides the method, the size of the sample will impact the accuracy of the estimate in a similar way as the number of replicates will determine the accuracy of the experiment. How large the sample size needs to

TABLE 1 Different parameters, their formula and remarks for insect production experiments

Parameter	Formula	Remarks
Yield	Yield = EWL – IWL	The most important parameter for a production experiment, preferably provided on a dry weight basis or provide both wet yield and dry weight%.
Dry weight (DW)	$DW\,(\%) = \frac{\text{Dry weight(g)}}{\text{Fresh weight (g)}} \times 100$	Including dry weight content (%) in all publications is essential for ensuring comprehensive and comparable reporting of collected data.
Feed conversion ratio (FCR)	$FCR = \frac{IS}{Yield}$	It is recommended to express the FCR in dry weight over weight (dry substrate and dry insect biomass) given that the live weight of larvae can differ based on the water content of the substrate. When the substrate is waste, it can be referred to as waste conversion ratio (WCR). Lower number indicate a more efficient conversion.
Bioconversion rate (BCR)	$BCR = \frac{Yield}{IS}$	The amount of larval biomass produced per unit of substrate and is recommended to express in% dry weight. Higher values indicate more efficient conversion.
Efficiency of conversion index (ECI)	$ECI = \frac{Yield}{IS–LS}$	See FCR, but conversely higher values indicate more efficient conversion. Estimating the leftover substrate at the end is difficult or impossible as it is mixed with the frass.
Waste reduction (WR)	$WRI\,(\%) = \frac{\text{IS(g)–Frass (g)}}{\text{IS (g)}} \times 100$	Mostly used in the context of using the insect bioconversion process as a mean for waste management. The amount of waste reduced over period of time, typically higher WR indicates more efficient waste processed by the larvae.
Mortality rate (MR)	$MR\,(\%) = \frac{\text{No. of larvea start–No. larvae end}}{\text{No. larvae start}} \times 100$	Usually, dead larvae cannot be found by the end of the experiment as they can be consumed by other larvae. Mortality can only be estimated if the initial number of larvae also were estimated. Escaped larvae (or killed by wrong handling methods), will also be attributed to mortality.
Survival rate (SR)	$SR\,(\%) = \frac{\text{No. larvae end}}{\text{No. larvae start}} \times 100$	Either survival rate or mortality rate should be provided.
Protein efficiency ratio (PER)	$PER = \frac{\text{protein Yield}}{\text{protein IS(g)}}$	Commonly used in animal experiments as an indicator of nutritional quality of food proteins. Can be adapted to any other element, or nutrient in the substrate (e.g. carbon conversion ratio).
Specific growth rate (SGR)	$SGR\,(\%/day) = \frac{\ln(EWL)–\ln(IWL)}{\text{Duration of trial}}$	In the formula the natural logarithm, ln, is used. A higher value is an indicator of high growth rate.

IS, initial substrate weight; LS, leftover substrate weight; EWL, end weight of the larvae; IWL, initial weight of the larvae.

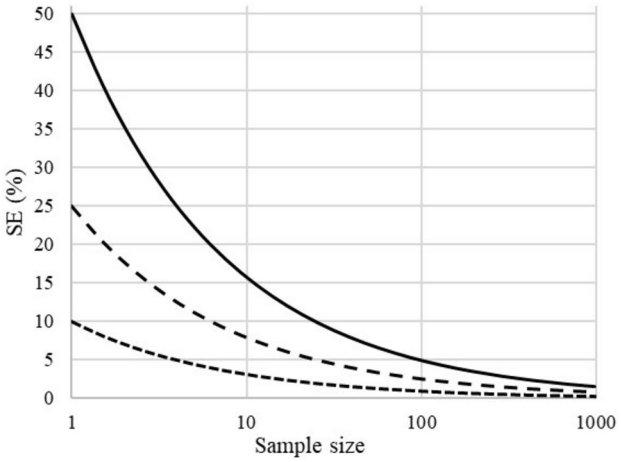

FIGURE 1 A visualisation between the relation of the standard error% ((SE/mean) × 100) and the sample size for a population with a 50% (solid line), 25% (dashed line) and 10% standard deviation (dotted line).

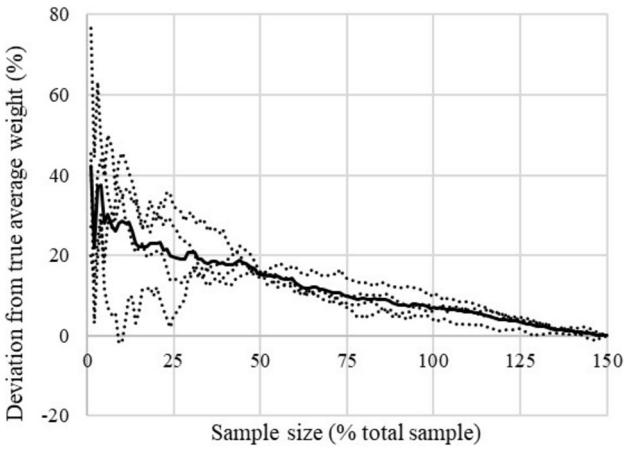

FIGURE 2 The deviation in% between the measured and true average weight of this population of MW larvae (total population 150) compared to the sample size when picked by hand. The dotted lines are the individual batches, the solid line the average of the four batches.

be depends on the differences you want to observe and the variability in the parameter (e.g. individual larvae size). Theoretically, if all larvae would be identical then a single larvae would be enough to know the true value, but this is not the case in real life. As a rule of thumb, we suggest using samples with at least 100 larvae. In a sample with a lot of variation between larvae sizes (e.g. CoV of 50%) this would lead to a parameter estimate (95% of the time) just below 10% of the true value of that parameter in a random sample. This is calculated as 1.96 times the standard error (SD/square root of the sample size or 50/10). In figure 1, the relation between the standard error and sample size for a population with an average of 100 and standard deviation of 10, 25 and 50 is presented to make this more visual and make informed decisions on minimum sample size.

It is also important to assess all larvae within the sample as we are inherently bad at taking a random sample by sight with a tendency to take larger individuals. As an example, real data is presented of four different batches of 150 MW larvae that were weighed individually by a student. It is important to note that the student was asked to do it *at random* but only had limited experience with insect larvae. The continuous average (each value is the average of all previous measurements) was calculated and compared to the true value. This is visualized in Figure 2. It is clear that, in nearly al cases, the measured average weight is higher than the actual average weight as the student was more likely to start with larger larvae. This effect can be significant as, as there is almost a 20% deviation from the real average if only the first 50 larvae would have been sampled and the others discarded. Only in one of the four replicates, the estimated average came close to the actual average in the first 30 measurements.

Population genetics

The target of virtually all above addressed aspects of experimental design for investigating production parameters is actually measuring insect phenotypes. Trait variation is influenced by several environmental factors but crucially also genetically determined. In contrast to huge efforts in comparative phenotyping, adequately addressing the genetic background of any given insect population under investigation has so far been largely neglected. Whereas not considering genetics as a factor has no influence for the interpretation of a given study using a single strain, ignoring such principally easy-to-generate metadata slows down the formation of "big pictures" on a global scale.

Although population genetic structures of most insects farmed for food and feed remain poorly investigated, our understanding of evolutionary and demographic relationships among global populations of the BSF (Generalovic *et al.*, 2023; Kaya *et al.*, 2021) and the MW (Eleftheriou *et al.*, 2022) is improving. Just like earlier research has helped to improve how insects are reared, making better use of their breeding potential could support the growth of the sector (Athanassiou *et al.*, 2024). Hence, tailoring existing concepts and tools for genetics and genomics research to the design and conduct of experimental insect production deserves stronger focus. For instance, Ståhls *et al.* (2020) and Kaya *et al.* (2021) imply that substantial genetic distances among geographically diversified lineages might explain some of the variation in production

TABLE 2 Recommended information to be added in a publication ensuring proper reproducibility is possible

Topic	Minimum recommended information	"If available/if applicable" information
Population and colony	· Origin of the population · Population age (years in rearing) · Substrate for population maintenance	· Life history traits (e.g. population developmental times) · Size of facility · Larvae density · Fly density
Climate	· Ambient temperature in °C (±variation) · Relative humidity in% (±variation) · Light/dark regime	· Ventilation information · In crate temperature °C · In crate pH
Substrate	· Weight of the substrate per crate (±variation) · Substrate composition (including ratios of ingredients) · Chemical composition (dry weight, protein, fat, ash, etc.) · Pretreatment and/or storage (freezing, drying, heating, shredding, etc.) · Feeding regime (single lump vs multiple rations)	· Chemical composition of the individual ingredients (dry weight, protein, fat, ash) · Particle size of the dry components
Housing	· Crate specifics (size, shape, material, surface area of the crate, etc.) · The use of a lid or other type of crate covering (netting) · The initial number of larvae and the quantification method	
Experiment and sample processing	· Age and weight of larvae at the start of the experiment (±variation) · Initial and final number of larvae (±variation) · Rearing period (days) · Harvest timepoint criteria · Harvesting method (manual sieving, washing, etc.) · Sampling methods (methodology, sample size, technical replicates, etc.) · Measurements (weight, number of larvae in the samples) · Sample processing (storage, drying, grinding, analyses, etc.)	· Visual inspections on the larval variations throughout the rearing (colour, curls, location, etc.)
Statistics (Smetana *et al.*, 2025)	· Statistical tests · Assumptions testing · Any data transformations or use of family/link functions · Model formulation and simplification · Program used and version	

traits. Indeed, in addition to expected dietary effects, surprisingly strong BSF genetic effects plus pronounced genotype-by-environment interactions were found for gut microbiome communities (Greenwood *et al.*, 2021), larval performance and composition (Generalovic *et al.*, 2025; Sandrock *et al.*, 2022).

However, solely reporting the geographic origin or laboratory/producer source is inconclusive for genetic discrimination and anecdotal origin stories are of little use for genetic discrimination in the light of frequent global trading (Kaya *et al.*, 2021; Ståhls *et al.*, 2020). Moreover, in small populations, the genetic makeup may severely change over time due to random

genetic drift and inbreeding (Hull *et al.*, 2024; Rhode *et al.*, 2020). In this sense, while phenotypic differences between insect strains within a shared experimental setup suggest genetic differences (Adamaki-Sotiraki *et al.*, 2022, 2023a,b, Rumbos *et al.*, 2020; Tognocchi *et al.*, 2024), verification thereof requires dedicated genetic profiling. To learn more about the various options for implementing genetic markers and analysis tools for a range of research questions and resource availabilities we refer to the BugBook article on genetics (Sandrock *et al.*, 2025). Exploring genotype-phenotype associations (Greenwood *et al.*, 2021; Hull *et al.*, 2023, 2024; Sandrock *et al.*, 2022) more rigorously is an opportunity for which, in the case of infrastructural constraints, qualified collaborations with academic or commercial partners are highly encouraged. In the end, similar to the impact made in conventional farmed animals, the economic efficiency of farmed insects can be improved (Zaalberg *et al.*, 2024) building on a solid population genetics context and systematic selective breeding (Facchini *et al.*, 2022; Hansen *et al.*, 2025, Sellem *et al.*, 2024).

Unintentional or unknown effects

Because of the design or execution of the experiment an abundance of (semi) unknown potential influences that can alter the results in unexpected ways may occur. For example, during substrate pretreatment: (1) If substrate is stored frozen before the start of the experiment this will not necessarily change the nutritional content thereof, but may change the structure of the substrate as ice crystals rupture the cell walls or (2) the substrate may heat up too much during blending or grinding operations, especially with dry substrate, altering the bioavailability of the nutrients, or (3) dust particles may face higher chance to disappear during blending, and slightly change nutrient composition. Although these unintentional effects may not always be avoidable, it is important to consider them and report the (pre)treatment steps.

A second example is the ventilation rate in the climate room. Ventilation is unavoidable as it is necessary to control the climate and air quality during an experiment. Nevertheless, the air exchange rate, air flow and its dynamics may alter the heat and evaporation from the crate and thereby the growth of the larvae. Two scenarios of how suboptimal ventilation may disrupt the experiment: (1) A lack of ventilation can result in the escape of BSF larvae if a reduced ventilation is paired with high moisture conditions within the crate. This causes a reduction of processing efficiency and biomass loss and ultimately bias in the results. (2) An inadequately high ventilation may lead to prematurely drying of the substrate before it is fully consumed, but a low ventilation rate may also slow down the evaporation too much, resulting in a difficult to process mix of frass and larvae at the end of the experiment (Cheng *et al.*, 2017).

A third example of unknown effects is the potential presence of insecticides, toxins, hormone analogues, and other chemical compounds in the substrate. Many toxins are not routinely checked due to the sheer number of them and for insecticides even values below the legal limit may impose subtle sublethal effects (Meijer *et al.*, 2021). In most cases, they are only sought and found after an experiment has failed. Similarly, while BSF larvae are found to effectively degrade mycotoxins in contaminated substrate, these compounds risk causing side effects on development (Heuel *et al.*, 2023, Niermans *et al.*, 2021) and should thus be prevented. All these difficult to control variables are the reason why the laboratory assessment and historical control (see Assessment of laboratory conditions) is important as deviations thereof may provide clues about unintentional issues.

5 Publication information

Table 2 provides recommended information to be added in a publication ensuring proper reproducibility is possible. Each journal typically provides a comprehensive author guideline that outlines the structure of a publication, however it does not go into detail on the specific data that needs to be provided in the material and methods part. In this section a set of experimental information and parameters to be included in a manuscript, serving as guidelines to ensure maximum repeatability of the study and facilitate future meta-analysis studies.

Acknowledgements

This article is part of the BugBook, initiated by the working group on Standardization of methods, parameters, and terminology in insect research of the European Federation of Animal Science (EAAP) insects commission. The work is supported by the German Ministry for Education and Research (BMBF) (CUBES project; grant number 031B0733A). C. Sandrock was supported through the "Innovation Space NewFoodSystems" (Project reKultI4Food) funded by BMBF (grant no. 031B1206A). This work was also supported by the EU-PRIMA program project ADVAGROMED (PRIMA

2021 – Section 2) and received partial financial support by CIPROMED (No. 2231, PRIMA Call 2022 Section 1 Agri-food IA) and the Flemish research foundation FWO SBO PetSect S001922N and the Swiss National Science Foundation and Swiss Agency for Development and Cooperation (SDC) under the Solution Oriented Research for Development Programme (SOR4D) under SWIFT project, Gran No. 400440_213241/1. The authors want to thank the reviewers, their efforts have greatly contributed to the improvement of this work.

References

Abduh, M.Y., Perdana, M.P., Bara, M.A., Anggraeni, L.W. and Putra, R.E., 2022. Effects of aeration rate and feed on growth, productivity and nutrient composition of black soldier fly (*Hermetia illucens* L.) larvae. Journal of Asia-Pacific Entomology 25: 101902. https://doi.org/10.1016/j.aspen.2022.101902

Adamaki-Sotiraki, C., Choupi, D., Vrontaki, M., Rumbos, C.I. and Athanassiou, C.G., 2024. Go local: Enhancing sustainable production of *Tenebrio molitor* through valorisation of locally available agricultural byproducts. Journal of Environmental Management 355: 120545.

Adamaki-Sotiraki, C., Deruytter, D., Rumbos, C.I. and Athanassiou, C.G., 2023a. Cross-breeding of *Tenebrio molitor* strains from a large-scale perspective. Journal of Insects as Food and Feed 10: 855-864. https://doi.org/10.1163/23524588-20230116

Adamaki-Sotiraki, C., Rumbos, C.I. and Athanassiou, C.G., 2022. Strain effect on the adult performance of the yellow mealworm, *Tenebrio molitor* L. Journal of Insects as Food and Feed 8: 1401-1410. https://doi.org/10.3920/JIFF2021.0207

Adamaki-Sotiraki, C., Rumbos, C.I. and Athanassiou, C.G., 2023b. Mating compatibility and offspring traits evaluation among different strains of *Tenebrio molitor*. Environmental Science and Pollution Research International 30: 97052-97062.

Agnew, J.M. and Leonard, J.J., 2003. The physical properties of compost. Compost Science and Utilization 11: 238-264.

Athanassiou, C.G., Coudron, C.L., Deruytter, D., Rumbos, C.I., Gasco, L., Gai, F., Sandrock, C., De Smet, J., Tettamanti, G., Francis, A., Petrusan, J.I. and Smetana, S., 2024. A decade of advances in black soldier fly research: from genetics to sustainability. Journal of Insects as Food and Feed 1: 1-28.

Auger, L., Tegtmeier, D., Caccia, S., Klammsteiner, T. and De Smet, J., 2025. BugBook: How to explore and exploit the insect-associated microbiome. Journal of Insects as Food and Feed 11: S361-S395. https://doi.org/10.1163/23524588-bja10256

Barragan-Fonseca, K.B., Dicke, M. and van Loon, J.J., 2018. Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). Entomologia Experimentalis et Applicata 166: 761-770.

Bekker, N.S., Heidelbach, S., Vestergaard, S.Z., Nielsen, M.E., Riisgaard-Jensen, M., Zeuner, E.J., Bahrndorff, S. and Eriksen, N.T., 2021. Impact of substrate moisture content on growth and metabolic performance of black soldier fly larvae. Waste Management 127: 73-79.

Bellezza Oddon, S., Biasato, I. and Gasco, L., 2022. Isoenergetic-practical and semi-purified diets for protein requirement determination in *Hermetia illucens* larvae: consequences on life history traits. Journal of Animal Science and Biotechnology 13: 17. https://doi.org/10.1186/s40104-021-00659-y

Bergman, T.L., 2011. Fundamentals of heat and mass transfer. John Wiley and Sons, Hoboken, NJ.

Biasato, I., Oddon, S.B., Loiotine, Z., Resconi, A. and Gasco, L., 2024. Wheat starch processing by-products as rearing substrate for black soldier fly: does the rearing scale matter?. Animal 18: 101238.

Bloukounon-Goubalan, A.Y., Saïdou, A., Chrysostome, C.A.A.M., Kenis, M., Amadji, G.L., Igué, A.M. and Mensah, G.A., 2020. Physical and chemical properties of the agro-processing by-products decomposed by larvae of Musca domestica and *Hermetia illucens*. Waste and Biomass Valorization 11: 2735-2743.

Boakye-Yiadom, K.A., Ilari, A. and Duca, D., 2022. Greenhouse gas emissions and life cycle assessment on the black soldier fly (*Hermetia illucens* L.). Sustainability 14: 10456.

Bosch, G., Oonincx, D.G.A.B., Jordan, H.R., Zhang, J., Van Loon, J.J.A., Van Huis, A. and Tomberlin, J.K., 2020. Standardisation of quantitative resource conversion studies with black soldier fly larvae. Journal of Insects as Food and Feed 6: 95-109.

Bruno, D., Bonelli, M., De Filippis, F., Di Lelio, I., Tettamanti, G., Casartelli, M., Ercolini, D. and Caccia, S., 2019. The intestinal microbiota of *Hermetia illucens* larvae is affected by diet and shows a diverse composition in the different midgut regions. Applied and Environmental Microbiology 85: e01864-18.

Charan, J. and Kantharia, N., 2013. How to calculate sample size in animal studies? Journal of Pharmacology and Pharmacotherapeutics 4: 303-306.

Cheng, J.Y., Chiu, S.L. and Lo, I.M., 2017. Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconver-

sion. Waste Management 67: 315-323. https://doi.org/10.1016/j.wasman.2017.05.046

Chia, S.Y., Tanga, C.M., Khamis, F.M., Mohamed, S.A., Salifu, D., Sevgan, S., Fiaboe, K.K.M., Niassy, S., van Loon, J.J.A., Dicke, M. and Ekesi, S., 2018. Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. PLoS ONE 13: e0206097.

Coudron, C.L., Deruytter, D., Craeye, S. and Bleyaert, P., 2022a. Entomoponics: combining insect rearing and greenhouse vegetable production – a case study with *Tenebrio molitor* and high-wire cucumber cultivation. Journal of Insects as Food and Feed 8: 427-438.

Coudron, C.L., Deruytter, D. and Claeys, J., 2022b. The influence of wet feed pH on the growth of *Tenebrio molitor* larvae. Sustainability 14: 7841. https://doi.org/10.3390/su14137841

Coudron, C.L., Berrens, S., Van Peer, M., Deruytter, D., Claeys, J. and Van Miert, S., 2024. Ammonia emissions related to black soldier fly larvae during growth on different diets. Journal of Insects as Food and Feed 1: 1-15. https://doi.org/10.1163/23524588-00001049

Coudron, C.L., Adamaki-Sotiraki, C., Yakti, W., Pascual, J.J., Wiklicky, V., Sandrock, C., Van Peer, M., Athanassiou, C., Peguero, D.A., Rumbos, D., Naser El Deen, S., Veldkamp, T., Deruytter, D. and Cambra-López, M., 2025. BugBook: Basic information and good practices on how to maintain stock populations for *Tenebrio molitor* and *Hermetia illucens* for research. Journal of Insects as Food and Feed 11: S241-S267. https://doi.org/10.1163/23524588-bja10240

Deruytter, D., Coudron, C.L. and Claeys, J., 2021. The influence of wet feed distribution on the density, growth rate and growth variability of *Tenebrio molitor*. Journal of Insects as Food and Feed 7: 141-150.

Deruytter, D., Coudron, C.L. and Claeys, J., 2022. The effects of density on the growth and temperature production of *Tenebrio molitor* larvae. Sustainability 14: 6234. https://doi.org/10.3390/su14106234

Deruytter, D., Coudron, C.L. and Claeys, J., 2023a. Transporting *Tenebrio molitor* eggs: the effect of temperature, humidity and time on the hatch rate. Sustainability 15: 6231.

Deruytter, D., Gasco, L., Yakti, W., Katz, H., Coudron, C.L., Gligorescu, A., Frooninckx, L., Noyens, I., Meneguz, M., Grosso, F., Bellezza Oddon, S., Biasato, I., Mielenz, M., Veldkamp, T., Van Loon, J.J.A., Spranghers, T., Vandenberg, G.W., Oonincx, D.G.A.B. and Bosch, G., 2023b. Standardising black soldier fly larvae feeding experiments: an initial protocol and variability estimates. Journal of Insects as Food and Feed 10: 1685-1696.

Deruytter, D., Rumbos, C.I., Adamaki-Sotiraki, C., Tournier, L., Ageorges, V., Coudron, C.L., Yakti, W., Ulrichs, C., Spranghers, T., Berrens, S., Van Peer, M., Bellezza Oddon, S., Biasato, I., Resconi, A., Paris, N., Hotte, N., Hénault-Ethier, L., Gasco, L. and Athanassiou, C.G., 2025. Make it a standard? The creation and variability assessment of a consensus standard protocol for *Tenebrio molitor* larvae feeding trials. Journal of Insects as Food and Feed 11: 1013-1033.

Dortmans, B.M.A., Diener, S., Verstappen, B.M. and Zurbrügg, C., 2017. Black soldier fly biowaste processing: a step-by-step guide. Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf.

Dzepe, D., Nana, P., Kuietche, H.M., Kimpara, J.M., Magatsing, O., Tchuinkam, T. and Djouaka, R., 2021. Feeding strategies for small-scale rearing black soldier fly larvae (*Hermetia illucens*) as organic waste recycler. SN Applied Sciences 3: 1-9.

Eberle, S., Schaden, L.M., Tintner, J., Stauffer, C. and Schebeck, M., 2022. Effect of temperature and photoperiod on development, survival, and growth rate of mealworms, *Tenebrio molitor*. Insects 13: 321.

Eleftheriou, E., Vacherie, B., Labadie, K., Athanassiou, C., Rigaud, T., Moret, Y., Lefebvre, T. and Madoui, M.-A., 2022b. *Tenebrio molitor* genomic structure among available populations. In: Proceedings of 12th World Congress on Genetics Applied to Livestock Production (WCGALP) Technical and species orientated innovations in animal breeding, and contribution of genetics to solving societal challenges. Wageningen Academic Publishers, Wageningen, pp. 2549-2551.

Ewusie, E.A., Kwapong, P.K., Ofosu-Budu, G., Sandrock, C., Akumah, A., Nartey, E., Teye-Gaga, C., Agyarkwah, S.A. and Adamtey, N., 2018. Development of black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae) in selected organic market waste fractions in Accra, Ghana. Asian Journal of Biotechnology and Bioresource Technology 4: 1-16.

Ewusie, E.A., Kwapong, P.K., Ofosu-Budu, G., Sandrock, C., Akumah, A., Nartey, E., Teye-Gaga, C. and Agyarkwah, S.A., 2019. The Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae): Trapping and culturing of wild colonies in Ghana. Scientific African 5: e00134. https://doi.org/10.1016/j.sciaf.2019.e00134

Facchini, E., Shrestha, K., Peeters, K. and Schmitt, E., 2022. Long-term artificial selection for increased larval body weight of *Hermetia illucens* in industrial settings. Frontiers in Genetics 13: 865490.

Frooninckx, L., Broeckx, L., Goossens, S., Wuyts, A. and Van Miert, S., 2024. Optimizing substrate moisture content for enhanced larval survival and growth performance in *Hermetia illucens*: exploring novel approaches. Discover Animals 1: 7.

Fuhrmann, A.J., Gold, M., Loh Ker, R., Chu, C.X., Haberkom, I., Puniamoorthy, N. and Mathys, A., 2024. Physical food waste properties alter rearing performance and respiration of black soldier fly larvae bioconversion. *XXVII International Congress of Entomolgy, Kyoto, Japan*.

Generalovic, T.N., Sandrock, C., Roberts, B.J., Meier, J.I., Hauser, M., Warren, I.A., Pipan, M., Durbin, R. and Jiggins, C.D., 2023. Cryptic diversity and signatures of domestication in the Black Soldier Fly (*Hermetia illucens*). bioRxiv: 2023-10.

Generalovic, T.N., Sandrock, C., Leonard, S., Schuldiner-Harpaz, T., Pipan, M., Welch, J.J. and Jiggins, C.D., 2025. Variation in strain performance and estimates of heritability of body size indicate considerable potential for genetic improvement of the black soldier fly (*Hermetia illucens*). Entomologia Experimentalis et Applicata 173: 558-574.

Gligorescu, A., Toft, S., Hauggaard-Nielsen, H., Axelsen, J.A. and Nielsen, S.A., 2018. Development, metabolism and nutrient composition of black soldier fly larvae (*Hermetia illucens*; Diptera: Stratiomyidae) in relation to temperature and diet. Journal of Insects as Food and Feed 4: 123-133.

Gold, M., Binggeli, M., Kurt, F., de Wouters, T., Reichlin, M., Zurbrügg, C., Mathys, A. and Kreuzer, M., 2020. Novel experimental methods for the investigation of *Hermetia illucens* (Diptera: Stratiomyidae) larvae. Journal of Insect Science 20: 21.

Gold, M., Tomberlin, J.K., Diener, S., Zurbrügg, C. and Mathys, A., 2018. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: A review. Waste Management 82: 302-318.

Gorrens, E., Lecocq, A. and De Smet, J., 2023. The use of probiotics during rearing of *Hermetia illucens*: potential, caveats, and knowledge gaps. Microorganisms 11(2): 245.

Greenwood, M.P., Hull, K.L., Brink-Hull, M., Lloyd, M. and Rhode, C., 2021. Feed and host genetics drive microbiome diversity with resultant consequences for production traits in mass-reared black soldier fly (*Hermetia illucens*) larvae. Insects 12: 1082.

Gross, A.J. and Mantel, N., 1967. The effective use of both positive and negative controls in screening experiments. Biometrics 23: 285-295.

Guillaume, J.B., Mezdour, S., Marion-Poll, F., Terrol, C. and Schmidely, P., 2023. Asymptotic estimated digestibility, a new indicator of black soldier fly (*Hermetia illucens*) conversion efficiency in relation to larval density. Journal of Insects as Food and Feed 9: 893-906.

Gulsunoglu-Konuskan, Z. and Dag, S., 2024. Physicochemical properties and ellagic acid accumulation in *Tenebrio molitor* larvae fed with pomegranate peel-enriched media. European Food Research and Technology 250: 1473-1483.

Hansen, L.L., Ramløv, H. and Westh, P., 2004. Metabolic activity and water vapour absorption in the mealworm *Tenebrio molitor* L.(Coleoptera, Tenebrionidae): real-time measurements by two-channel microcalorimetry. Journal of Experimental Biology 207(3): 545-552.

Hansen, L.S., Laursen, S.F., Bahrndorff, S., Sørensen, J.G., Sahana, G., Kristensen, T.N. and Nielsen, H.M., 2025. The unpaved road towards efficient selective breeding in insects for food and feed – a review. Entomologia Experimentalis et Applicata 173: 498-521.

Heuel, M., Kreuzer, M., Sandrock, C., Leiber, F., Mathys, A., Guggenbühl, B., Gangnat, I.D.M. and Terranova, M., 2022. Feeding value of black soldier fly larvae compared to soybean in methionine- and lysine-deficient laying hen diets. Journal of Insects as Food and Feed 8: 989-999. https://doi.org/10.3920/JIFF2021.0178

Heuel, M., Kreuzer, M., Gangnat, I.D.M., Frossard, E., Zurbrügg, C., Egger, J., Dortmans, B., Gold, M., Mathys, A., Jaster-Keller, J., Weigel, S., Sandrock, C. and Terranova, M., 2023. Low transfer of cadmium, lead and aflatoxin B1 to eggs and meat of laying hens receiving diets with black soldier fly larvae reared on contaminated substrates. Animal Feed Science and Technology 304: 115733. https://doi.org/10.1016/j.anifeedsci.2023.115733

Hill, V., Lopez-Viso, C., Brameld, J., Salter, A. and Parr, T., 2024. The juvenile hormone analogue, pyriproxifen, alters protein and fat composition of *Tenebrio molitor* larvae. Journal of Insects as Food and Feed 10: 1633-1644.

Holmes, L.A., Vanlaerhoven, S.L. and Tomberlin, J.K., 2012. Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). Environmental Entomology 41: 971-978.

Hull, K.L., Greenwood, M.P., Lloyd, M., Bester-van der Merwe, A.E. and Rhode, C., 2023. Gene expression differentials driven by mass rearing and artificial selection in black soldier fly colonies. Insect Molecular Biology 32: 86-105.

Hull, K.L., Greenwood, M.P., Lloyd, M., Brink-Hull, M., Bester-van der Merwe, A.E. and Rhode, C., 2024. Drivers of genomic diversity and phenotypic development in early phases of domestication in *Hermetia illucens*. Insect Molecular Biology 33: 756-776.

Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54: 187-211.

Ingram, T., 2018. Evaluating the feasibility of using screw conveyors as a means to continuously grow black soldier fly larvae. Doctoral dissertation, MIT, Cambridge, MA.

Johnsen, N.S., Andersen, J.L. and Offenberg, J., 2021. The effect of relative humidity on the survival and growth rate of the yellow mealworm larvae (*Tenebrio molitor*, Linnaeus 1758). Journal of Insects as Food and Feed 7: 311-318.

Kaya, C., Generalovic, T.N., Ståhls, G., Hauser, M., Samayoa, A.C., Nunes-Silva, C.G., Roxburgh, H., Wohlfahrt, J., Ewusie,

E.A., Kenis, M., Hanboonsong, Y., Orozco, J., Carrejo, N., Nakamura, S., Gasco, L., Rojo, S., Tanga, C.M., Meier, R., Rhode, C., Picard, C.J., Jiggins, C., Leiber, F., Tomberlin, J.K., Hasselmann, M., Blanckenhorn, W.U., Kapun, M. and Sandrock, C., 2021. Global population genetic structure and demographic trajectories of the black soldier fly, *Hermetia illucens*. BMC Biology 19: 94. https://bmcbiol.biomedcentral.com/articles/10.1186/s12915-021-01029-w

Ko, H., Cassidy, G.J., Shishkov, O., Aydin, E., Hu, D.L. and Goldman, D.I., 2021. Air-fluidized aggregates of black soldier fly larvae. Frontiers in Physics 9: 734447.

Kotsou, K., Chatzimitakos, T., Athanasiadis, V., Bozinou, E., Athanassiou, C.G. and Lalas, S.I., 2023. Innovative applications of *Tenebrio molitor* larvae in food product development: a comprehensive review. Foods 12: 4223.

Kotsou, K., Chatzimitakos, T., Athanasiadis, V., Bozinou, E. and Lalas, S.I., 2024. Exploiting agri-food waste as feed for *Tenebrio molitor* larvae rearing: a review. Foods 13: 1027.

Laksanawimol, P., Anukun, P. and Thancharoen, A., 2024. Use of different dry materials to control the moisture in a black soldier fly (*Hermetia illucens*) rearing substrate. PeerJ 12: e17129.

Lalander, C., Ermolaev, E., Wiklicky, V. and Vinnerås, B., 2020. Process efficiency and ventilation requirement in black soldier fly larvae composting of substrates with high water content. Science of the Total Environment 729: 138968.

Laursen, S.F., Flint, C.A., Bahrndorff, S., Tomberlin, J.K. and Kristensen, T.N., 2024. Reproductive output and other adult life-history traits of black soldier flies grown on different organic waste and by-products. Waste Management 181: 136-144.

Lemke, N.B., Dickerson, A.J. and Tomberlin, J.K., 2023. No neonates without adults: A review of adult black soldier fly biology, *Hermetia illucens* (Diptera: Stratiomyidae). BioEssays 45: 2200162.

Li, C., Addeo, N.F., Rusch, T.W., Tarone, A.M. and Tomberlin, J.K., 2023. Black soldier fly (Diptera: Stratiomyidae) larval heat generation and management. Insect Science 30: 964-974.

Liu, X., Chen, X., Wang, H., Yang, Q., ur Rehman, K., Li, W., Cai, M., Mazza, L., Zhang, J., Yu, Z. and Zheng, L., 2017. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. PLoS ONE 12: e0182601.

Loiotine, Z., Gasco, L., Biasato, I., Resconi, A. and Bellezza Oddon, S., 2024. Effect of larval handling on black soldier fly life history traits and bioconversion efficiency. Frontiers in Veterinary Science 11: 1330342.

Lopes, I.G., Wiklicky, V., Ermolaev, E. and Lalander, C., 2023. Dynamics of black soldier fly larvae composting – Impact of substrate properties and rearing conditions on process efficiency. Waste Management 172: 25-32.

McEachern, T., 2018. Determining heat production of black soldier fly larvae, *Hermetia illucens*, to design rearing structures at livestock facilities. Master's thesis, University of Kentucky, Lexington, KY.

Megido, R.C., Francis, F., Haubruge, E., Le Gall, P., Tomberlin, J.K., Miranda, C.D., Jordan, H.R., Picard, C.J., Pino, M.J.M., Ramos-Elordy, J.R., Katz, E., Barragan-Fonseca, K.B., Costa-Neto, E.M., Ponce-Reyes, R., Wijffels, G., Ghosh, S., Jung, C., Han, Y.S., Conti, B., Vilcinskas, A., Tanga, C.M., Kababu, M.O., Beesigamukama, D., Morales Ramos, J.A. and van Huis, A., 2024. A worldwide overview of the status and prospects of edible insect production. Entomologia Generalis 44: 3-27.

Meijer, N., de Rijk, T., van Loon, J.J., Zoet, L. and Van der Fels-Klerx, H.J., 2021. Effects of insecticides on mortality, growth and bioaccumulation in black soldier fly (*Hermetia illucens*) larvae. PLoS ONE 16: e0249362.

Meneguz, M., Gasco, L. and Tomberlin, J.K., 2018. Impact of pH and feeding system on black soldier fly (*Hermetia illucens*, L; Diptera: Stratiomyidae) larval development. PLoS ONE 13: e0202591.

Meng, Y., Zhang, X., Zhang, Z., Li, J., Zheng, P., Li, J., Xu, J., Xian, J. and Lu, Y., 2023. effects of microorganisms on growth performance, body composition, digestive enzyme activity, intestinal bacteria flora and antimicrobial peptide (AMP) content of black soldier fly larvae (*Hermetia illucens*). Animals 13: 2722.

Mertenat, A., Diener, S. and Zurbrügg, C., 2019. Black soldier fly biowaste treatment – assessment of global warming potential. Waste Management 84: 173-181. https://doi.org/10.1016/j.wasman.2018.11.040

Morales-Ramos, J.A., Kay, S., Rojas, M.G., Shapiro-Ilan, D.I. and Tedders, W.L., 2015. Morphometric analysis of instar variation in *Tenebrio molitor* (Coleoptera: Tenebrionidae). Annals of the Entomological Society of America 108: 146-159.

Morales-Ramos, J.A., Rojas, M.G., Kelstrup, H.C. and Emery, V., 2020. Self-selection of agricultural by-products and food ingredients by *Tenebrio molitor* (Coleoptera: Tenebrionidae) and impact on food utilization and nutrient intake. Insects 11: 827.

Naser El Deen, S., Spranghers, T., Baldacchino, F. and Deruytter, D., 2022. The effects of the particle size of four different feeds on the larval growth of *Tenebrio molitor* (Coleoptera: Tenebrionidae). European Journal of Entomology 119: 242-249.

Nawoya, S., Ssemakula, F., Akol, R., Geissmann, Q., Karstoft, H., Bjerge, K., Mwikirize, C., Katumba, A. and Gebreyesus, G., 2024. Computer vision and deep learning in insects for food and feed production: a review. Computers and Electronics in Agriculture 216: 108503.

Niermans, K., Meyer, A.M., den Hil, E.H.V., Van Loon, J.J.A. and Van der Fels-Klerx, H.J., 2021. A systematic literature review on the effects of mycotoxin exposure on insects and on mycotoxin accumulation and biotransformation. Mycotoxin Research: 1-17.

Niu, S.H., Liu, S., Deng, W.K., Wu, R.T., Cai, Y.F., Liao, X.D. and Xing, S.C., 2022. A sustainable and economic strategy to reduce risk antibiotic resistance genes during poultry manure bioconversion by black soldier fly *Hermetia illucens* larvae: larval density adjustment. Ecotoxicology and Environmental Safety 232: 113294.

Oonincx, D.G.A.B., Van Broekhoven, S., van Huis, A. and Van Loon, J.J.A., 2015. Feed conversion, survival and development, andcomposition of four insect species on diets composed of food by-products. PLoS ONE 10: e0144601. https://doi.org/10.1371/journal.pone.0144601

Padmanabha, M., Kobelski, A., Hempel, A.J. and Streif, S., 2023. Modelling and optimal control of growth, energy, and resource dynamics of *Hermetia illucens* in mass production environment. Computers and Electronics in Agriculture 206: 107649.

Palma, L., Ceballos, S.J., Johnson, P.C., Niemeier, D., Pitesky, M. and VanderGheynst, J.S., 2018. Cultivation of black soldier fly larvae on almond byproducts: impacts of aeration and moisture on larvae growth and composition. Journal of the Science of Food and Agriculture 98: 5893-5900.

Parodi, A., Gerrits, W.J., Van Loon, J.J., De Boer, I.J., Aarnink, A.J. and Van Zanten, H.H., 2021. Black soldier fly reared on pig manure: Bioconversion efficiencies, nutrients in the residual material, greenhouse gas and ammonia emissions. Waste Management 126: 674-683.

Parra Paz, A.S., Carrejo, N.S. and Gómez Rodríguez, C.H., 2015. Effects of larval density and feeding rates on the bioconversion of vegetable waste using black soldier fly larvae *Hermetia illucens* (L.), (Diptera: Stratiomyidae). Waste and Biomass Valorization 6: 1059-1065.

Parsa, S.H., Kheiri, F., Fathipour, Y., Imani, S. and Chamani, M., 2023. Yellow mealworm (*Tenebrio molitor* L.) development time of life stages duration and survival rate at different temperatures in laboratory conditions. Arthropods 12: 16.

Peguero, D.A., Gold, M., Endara, A., Niu, M., Zurbrügg, C. and Mathys, A., 2023. Evaluation of ammonia pretreatment of four fibrous biowastes and its effect on black soldier fly larvae rearing performance. Waste Management 160: 123-134.

Peguero, D.A., Gold, M., Velasquez, L., Niu, M., Zurbrügg, C. and Mathys, A., 2024. Physical pretreatment of three biowastes to improve black soldier fly larvae bioconversion efficiency. Waste Management 178: 280-291.

Peng, C., Zhou, T., Song, S. and Sun, S., 2022. Analysis and experiment of feeding material process of *Hermetia illucens* L. frass bucket wheel based on DEM. Computers and Electronics in Agriculture 196: 106855.

Penning de Vries, B.B. and Groenwold, R.H., 2023. Negative controls: concepts and caveats. Statistical Methods in Medical Research 32: 1576-1587.

Ramanzin, M., Bailoni, L. and Bittante, G., 1994. Solubility, water-holding capacity, and specific gravity of different concentrates. Journal of Dairy Science 77: 774-781.

Resconi, A., Bellezza Oddon, S., Ferrocino, I., Loiotine, Z., Caimi, C., Gasco, L. and Biasato, I., 2024. Effects of brewery by-products on growth performance, bioconversion efficiency, nutritional profile, and microbiota and mycobiota of black soldier fly larvae. Animal 18: 101288.

Rhode, C. and Greenwood, M.P., 2023. Antimicrobial peptide gene expression and the microbiome in black soldier fly. Insect Sci 30: 1017-1021. https://doi.org/10.1111/1744-7917.13177

Ribeiro, N., Abelho, M. and Costa, R., 2018. A review of the scientific literature for optimal conditions for mass rearing *Tenebrio molitor* (Coleoptera: Tenebrionidae). Journal of Entomological Science 53: 434-454.

Rossi, G., Ojha, S., Berg, W., Herppich, W.B. and Schlüter, O.K., 2024. Estimating the dynamics of greenhouse gas emission during black soldier fly larvae growth under controlled environmental conditions. Journal of Cleaner Production 470: 143226, https://doi.org/10.1016/j.jclepro.2024.143226

Rumbos, C.I., Oonincx, D.G.A.B., Karapanagiotidis, I.T., Vrontaki, M., Gourgouta, M., Asimaki, A., Mente, E. and Athanassiou, C.G., 2021. Agricultural byproducts from Greece as feed for yellow mealworm larvae: circular economy at local level. Journal of Insects as Food and Feed 8: 9-22. https://doi.org/10.3920/JIFF2021.0044

Sandrock, C., Leupi, S., Wohlfahrt, J., Kaya, C., Heuel, M., Terranova, M., Blanckenhorn, W.U., Windisch, W., Kreuzer, M. and Leiber, F., 2022. Genotype-by-diet interactions for larval performance and body composition traits in the black soldier fly, *Hermetia illucens*. Insects 13: 424. https://doi.org/10.3390/insects13050424

Sandrock, C., Generalovic, T.N., Paul, K., Petersen, G.E.L., Sellem, E., Smith, M.B., Tapio, M., Yakti, W., Beukeboom, L.W., Deruytter, D., Jiggins, C.D., Lefebvre, T., Librado, P., Pannebakker, B.A., Picard, C.J., Rhode, C., Sorensen, J.G., Bouwman, A.C., Hansen, L.S. and Obšteter, J., 2025. BugBook: Genetics of insects as food and feed. Journal of Insects as Food and Feed 11: S397-S455. https://doi.org/10.1163/23524588-bja10260

Scala, A., Cammack, J.A., Salvia, R., Scieuzo, C., Franco, A., Bufo, S.A., Tomberlin, J.K. and Falabella, P., 2020. Rearing substrate impacts growth and macronutrient composition of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) lar-

vae produced at an industrial scale. Scientific Reports 10: 19448.

Schøn, M.L., Mikkelsen, M.V.N., Jensen, K., Poulsen, J.M., Berggreen, I.E., Schou, T.M., Nørgaard, J.V. and Overgaard, J., 2025. Effect of temperature on growth, metabolism, and gas exchange in *Hermetia illucens* larvae reared under commercial and laboratory conditions. Journal of Insects as Food and Feed 11: 1059-1074. https://doi.org/10.1163/23524588-00001268

Schreven, S.J., de Vries, H., Hermes, G.D., Zeni, G., Smidt, H., Dicke, M. and Van Loon, J.J., 2022. Black soldier fly larvae influence internal and substrate bacterial community composition depending on substrate type and larval density. Applied and Environmental Microbiology 88: e00084-22.

Sellem, E., Paul, K., Donkpegan, A., Li, Q., Masseron, A., Chauveau, A., Gagnepain-Germain, F. and Lefebvre, T., 2024. Multitrait genetic parameter estimates in a *Tenebrio molitor* reference population: high potential for breeding gains. Animal 18: 101197. https://doi.org/10.1016/j.animal.2024.101197

Shishkov, O., Hu, M., Johnson, C. and Hu, D.L., 2019. Black soldier fly larvae feed by forming a fountain around food. Journal of The Royal Society Interface 16: 20180735.

Shumo, M., Khamis, F.M., Tanga, C.M., Fiaboe, K.K., Subramanian, S., Ekesi, S., van Huis, A. and Borgemeister, C., 2019. Influence of temperature on selected life-history traits of black soldier fly (*Hermetia illucens*) reared on two common urban organic waste streams in Kenya. Animals 9: 79.

Smetana, S., Coudron, C., Deruytter, D., Francis, A., Pascual, J.J., Klammsteiner, T., Lemke, N., Sandrock, C. and Zanoli, R., 2025. BugBook: Data analysis methods in studies of insects for food and feed. Journal of Insects as Food and Feed 1(aop): 1-27. https://doi.org/10.1163/23524588-bja10209

Smets, R., Sibinga, N.A., Verheyen, G., Rossi, G. and Van Der Borght, M., 2025. BugBook: Common pitfalls and practical recommendations for chemical analysis of insect biomass. Journal of Insects as Food and Feed 1(aop): 1-19. https://doi.org/10.1163/23524588-bja10238

Ståhls, G., Meier, R., Sandrock, C., Hauser, M., Šašić Zorić, L., Laiho, E., Aracil, A., Doderović, J., Badenhorst, R., Unadirekkul, P., Mohd Adom, N.A.B., Wein, L., Richards, C., Tomberlin, J.K., Rojo, S., Veselić, S. and Parvainen, T., The puzzling mitochondrial phylogeography of the black soldier fly (*Hermetia illucens*), the commercially most important insect protein species. BMC Evolutionary Biology 20: 60. https://doi.org/10.1186/s12862-020-01627-2

Tognocchi, M., Abenaim, L., Adamaki-Sotiraki, C., Athanassiou, G.C., Rumbos, I.C., Mele, M., Conti, M. and Conte, G., 2024. Effect of different diet composition on the fat profile of two different Black Soldier Fly larvae populations. Animal: 101205.

Tomberlin, J.K., Klammsteiner, T., Lemke, N., Yadav, P. and Sandrock, C., 2025. BugBook: Black soldier fly as a model to assess behaviour of insects mass produced as food and feed. Journal of Insects as Food and Feed 1(aop): 1-20. https://doi.org/10.1163/23524588-bja10225

Van Peer, M., Berrens, S., Noyens, I., Goossens, S., Coudron, C., Lau, T., Alvarez, C. and Van Miert, S., 2023. Testing side streams as substrate for insects. ValuSect. Available online at https://www.valusect.eu/node/64

Vrontaki, M., Adamaki-Sotiraki, C., Rumbos, C.I., Anastasiadis, A. and Athanassiou, C.G., 2024. Valorization of local agricultural byproducts as nutritional substrates for *Tenebrio molitor* larvae: a sustainable approach to alternative protein production. Environmental Science and Pollution Research: 1-9. https://doi.org/10.1007/s11356-024-33564-8

Wang, Y., Zhang, Y., Wang, J., Kang, C., Hu, G., Guo, Y., Chen, J., Yang, L. and Wang, Y., 2023. Temperature dependent development of black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) from Yangtze River Delta region of China. Journal of Asia-Pacific Entomology 26: 102163.

Weaver, D.K. and McFarlane, J.E., 1990. The effect of larval density on growth and development of *Tenebrio molitor*. Journal of Insect Physiology 36: 531-536.

Witriana, N.I., Ardyati, T. and Jatmiko, Y.D., 2023. The Use of probiotics in fermenting food wastes for production of black soldier fly larvae (*Hermetia illucens* L.; Diptera: Stratiomyidae). Berkala Penelitian Hayati Journal of Biological Researches 29: 99-105.

Wynants, E., Crauwels, S., Lievens, B., Luca, S., Claes, J., Borremans, A., Bruyninckx, L. and Van Campenhout, L., 2017. Effect of post-harvest starvation and rinsing on the microbial numbers and the bacterial community composition of mealworm larvae (*Tenebrio molitor*). Innovative Food Science and Emerging Technologies 42: 8-15.

Wynants, E., Frooninckx, L., Crauwels, S., Verreth, C., De Smet, J., Sandrock, C., Wohlfahrt, J., Van Schelt, J., Depraetere, S., Lievens, B., Van Miert, S., Claes, J. and Van Campenhout, L., 2019. Assessing the microbiota of black soldier fly larvae (*Hermetia illucens*) reared on organic waste streams on four different locations at laboratory and large scale. Microbial Ecology 77: 913-930. https://doi.org/10.1007/s00248-018-1286-x

Yakti, W., Förster, N., Müller, M., Beck, S., Schulz, S., Mewis, I. and Ulrichs, C., 2025. Solid-state fermentation of hemp waste: enhancing the performance of *Hermetia illucens* larvae and altering the composition of hemp secondary metabolites. Frontiers in Bioengineering and Biotechnology 13: 1449233.

Yakti, W., Müller, M., Klost, M., Mewis, I., Dannehl, D. and Ulrichs, C., 2023. Physical properties of substrates as a driver for *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae growth. Insects 14: 266. https://doi.org/10.3390/insects14030266

Yakti, W., Schulz, S., Marten, V., Mewis, I., Padmanabha, M., Hempel, A.-J., Kobelski, A., Streif, S. and Ulrichs, C., 2022. The effect of rearing scale and density on the growth and nutrient composition of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae. Sustainability 14: 1772. https://doi.org/10.3390/su14031772

Yakti, W., Shaw, C., Müller, M., Mewis, I., Kloas, W. and Ulrichs, C., 2024. Tracing the journey of elements from fish feed to Nile tilapia faeces to black soldier fly larvae: a comparative approach. Frontiers in Sustainable Food Systems 8: 1298885.

Yang, F. and Tomberlin, J.K., 2020. Comparing selected life-history traits of black soldier fly (Diptera: Stratiomyidae) larvae produced in industrial and bench-top-sized containers. Journal of Insect Science 20(5): 25.

Zaalberg, R.M., Nielsen, H.M., Noer, N.K., Schou, T.M., Jensen, K., Thormose, S., Kargo, M. and Slagboom, M., 2024. A bio-economic model for estimating economic values of important production traits in the black soldier fly (*Hermetia illucens*). Journal of Insects as Food and Feed 10: 1411-1421. https://doi.org/10.1163/23524588-00001126