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RESEARCH ARTICLE

BugBook: Black soldier fly as a model to assess behaviour of insects mass produced as food and feed

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Abstract

Behavioural research and its applications has a rich history in science with direct applications continuing to expand global understanding of ecosystem function, structure, and evolution. The same can be said for such research as related to the applied sciences including entomology. The purpose of this chapter is to provide context to various approaches for assessing behaviour of insects that are mass produced for food and feed. By using the black soldier fly as a model, various approaches for conducting such research are explored along with providing some perspective on the value of such data for optimising insect production. However, it should be noted that this chapter is not exhaustive with regards to variables that can be examined, or the methods employed.

Keywords

choice test – electrophysiology – ethology – experimental design – reproduction

1 Introduction

Research in ethology as related to animal behaviour beyond humans is rarely straightforward. What determines behaviour can be simple or complex, emergent or chaotic, deterministic or stochastic. Moreover, behaviour is underpinned by several echelons of causal factors – from the environment and social context, to hormones, physiology (see Bruno *et al.*, 2025), gross cellular processes, genetics, epigenetics, and ultimately evolutionary history. The seminal work by Nikolaas Tinbergen; however, provides a simple structure for both describing and exploring the adaptive value of behavioural responses by posing four guiding questions (Tinbergen, 1963). Remarkably, besides the expansion into behavioural syndromes (i.e. suites of related behaviours in similar environmental contexts), Tinbergen's four questions still remain a key framework governing research today including the expansion into genetics.

These questions fall into two key categories, proximate (i.e. occurring recent) and ultimate (i.e. occurring in the distant past). The proximate questions are, a) causation, what is the mechanism, and b) developmental (i.e. ontogeny, how does the behaviour change over the lifetime of the animal. While the ultimate questions are, c) function, what is the adaptive significance as related to fitness, and d) evolution, how is the behaviour rep-

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resented at a phylogenetic scale? Critically, while each question can be examined individually, the intent is to view them in a holistic framework, since they are informative of one another. In fact, it is highly recommended Tinbergen's four question framework be applied as a means to unify research across systems and streamline terminology under the four umbrellas of research that he defined. Although, some researchers have also suggested adding a fifth question to modernise the framework — for example, what does an organism perceive, experience, value, or feel? (Dawkins, 2023) — doing so has yet to be universally accepted. Instead, Tinbergen's four original questions continue to principally guide behavioural research.

While the specific aims proposed by Tinbergen will not be referenced throughout this chapter, they will be integrated as part of the 'spirit' of each section. Regardless, we highly encourage that those interested in behavioural research review his work from 1963. Hence, the goal of this chapter is to discuss the various methods utilised to explore behavioural responses of insects within the food and feed sector. The discussed methods will cross levels of responses ranging from the individual, group being tested, and population from which they originate, as well as the genetic variation (e.g., context-dependent gene regulation). Of course, each of these can be its own chapter or even book; thus, it is important to note that the chapter provides an overview that can serve as a platform from which research can then expand into their respective realms.

2 Experimental intent in behavioural research

In scientific research, experimental intent is the fundamental component that shapes not only the design, execution, and analytical framework of the experiment but also the interpretation of the results and the conclusions drawn. In a field such as insect farming, with its global scale-up driven by industrialisation in the last decade (Athanassiou *et al.*, 2024), where basic and applied research is occurring in parallel under different settings, the clarity and specificity of experimental intention guides the whole research process from shaping the questions posed, research methodologies employed, and ultimately the application of the results. In this section, we delve into the role of experimental intent in behavioural research in adult black soldier flies, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), where intentions can range from understanding ultimate causes underlying biological processes by focusing on fundamental research to maximising production output. These goals can be accomplished in part by optimising the breeding and rearing conditions as well as welfare by focusing on proximate causes of behaviours. Clearly defining the objective becomes crucial as it informs every aspect of the experimental design, hypothesis formulation, as well as data collection and analysis.

Influence on experimental design

1. Selection of controls and variables: The intention of the experiment dictates the variables that need to be measured, and the controls required to ensure validity.
2. Methodology: The methodologies chosen are directly influenced by the experimental intention. Behavioural studies on black soldier flies might employ direct observation, video recording, or automated tracking systems, depending on whether the focus is on qualitative or quantitative data.
3. Experimental setting: Controlled laboratory conditions are often used to minimise external variables, while field studies might be necessary to observe natural behaviours.

Impact on outcomes and interpretation

1. Data analysis and interpretation: The intention shapes how data are analysed and interpreted. Specific hypotheses lead to targeted statistical tests and analytical methods.
2. Relevance and applicability: The outcomes of an experiment are considered most relevant when they directly address the initial research intention.
3. Generalisability and limitations: The specificity of the experimental intention also defines the scope and limitations of the findings. Highly specific intentions might yield detailed insights but could limit the generalisability of the results. Conversely, broader intentions may provide more general insights but might lack depth.

3 Environment

Defining the parameters for investigating behavioural responses of a target animal, such as black soldier fly adults or larvae, is critical for creating a framework in behavioural ecology that addresses a specific hypothesis. How animals respond in an artificial environment (e.g., adults in a cage in a factory, provided an artificial diet) versus a natural habitat (e.g., flies attracted to decomposing fruit in a forest) can be quite different and often difficult to translate across contexts. Basically, data from an industrial environment may provide limited if any inference for what occurs in natural habitats and vice versa. This then necessarily requires comparative studies to be conducted between wild and captive populations. With that said, a boon of conducting research in artificial systems is that system complexity is generally minimised relative to natural environments, such as by reducing or eliminating many of the factors that contribute to variability. Such factors include the provisioning of artificial diet and water (often *ad libitum*), providing amiable environmental conditions and refuges from intraspecific competition, the elimination of predation pressure and interspecies competition, as well as differential maternal imprinting, and, lastly, controlling immigration/emigration rates through standard-operating procedures. Thus, conducting behavioural work on captive populations should ideally be less challenging than doing so in a natural setting, which conversely involves population dynamics and uncontrolled environmental conditions.

The purpose of this section is to review some factors at play in either scenario and discuss how they may impact behavioural responses. This is a first step in order to make comparisons across these varying contexts. The intent will be to discuss abiotic and biotic factors in the broadest sense as they possibly impact all stages of insect development. We highlight the following: (1) that patterns are not easily translatable across scale; (2) behavioural patterns often are specific to populations, owing to differing demography, including evolutionary history and (intended or not) selection in captivity; and (3) that results can differ depending on methods. That said, comparisons can still be drawn between behaviour observed in different settings through abstraction. For example, while apples and oranges are often said to be fundamentally different, both are round edible fruits, meaning that their volume, nutrition, etc., can be quantified and compared. The same thinking can be applied to behaviour, which can often be connected through mathematical models or simulations (Kobelski *et al.*, 2024).

First, scale is a foundational concept that impacts all research. In the case of the black soldier fly, how adult flies operate within the confines of a 2 m^3 cage can be quite different than what occurs in an even smaller 30 × 30 × 30 cm cage (Nakamura *et al.*, 2016) simply due to its natural biology with lekking (e.g., aerial displays prior to mating) that can occur over large swaths of space such as a tree or large blankets of kudzu (Lemke *et al.*, 2023; Tomberlin and Sheppard, 2001). Consequently, the scale to be considered in a study should be one of the first parameters defined. Is the scale being tested relevant to the behaviour of interest? Could it be relevant for one behaviour but not another? Likewise, adult density is an important factor (Liu *et al.*, 2022), yet its relative relevance at different scales is poorly understood. As will be discussed in other sections, the answers will typically also depend on abiotic factors, and even 24 m^3 mating chambers may only partly better mimic natural conditions while still revealing specific constraints (Salari and De Goede, 2023). When working on a large scale (i.e. bigger than a 30 × 30 × 30 cm cage), microclimates are at play. For example, pockets of temperature, humidity, or light variation could result in segregation of individuals based on sex, size, and age (Salari and De Goede, 2024). The fragmentation of insects into groups can lead observed results to be determined by a specific subset of the population. Certainly, 'effective population size', *Ne*, is a metric used in population genetics to gauge the proportion of individuals that actually contribute to reproduction (see Sandrock *et al.*, 2025); the same thinking should be used for behaviour. That is, not all individuals within the population will exhibit the same tendencies.

Second, defining the population (size, genetic structure, dynamics) being examined is also critical for conducting behavioural research (Wilson and Bossert 1971). Population genetics is a fundamental aspect that generally deserves more rigorous attention in black soldier fly research. The black soldier fly is characterised by a huge gamma genetic diversity and global populations are highly structured according to biogeographic regions and contemporary farming activity (Generalovic *et al.*, 2023; Kaya *et al.*, 2021; Ståhls *et al.*, 2020). Genotype effects, including interactions with diet and microbiota, have been demonstrated for larval phenotypes (Greenwood *et al.*, 2021, Sandrock *et al.*, 2022, Silvaraju *et al.*, 2024, Zhang *et al.*, 2024) and likewise genetically differentiated populations are expected to display behavioural variation due to divergent ecological adaptation as well as artificial selection. Additionally, effects of nutritional history were shown to affect adult reproductive strategy (Laursen *et al.*, 2024). Moreover, pathogen load, prior experiences, and 'personality' of individuals can impact emergent behavioural responses at the level of the population (Leadbeater and Chittka, 2007). Understanding these factors is applicable both within industrial or natural environments and need to be considered as they have been known to impact adult longevity, mate choice, fertility, fecundity, and other life-history traits through bottom-up processes (Marsh, 2009). Moreover, understanding behaviour though the lens of emergent phenomena is especially important considering many are reared at high population densities or are otherwise gregarious in nature.

Lastly, understanding the connection between these factors and their impact on behavioural responses is critical for drawing proper conclusions from any data generated. Likewise, the methods themselves can create artefacts and bias interpretation. Whether or not to consider these factors holistically, or in a reduced (but tractable) system can also sway results. At the most basic level, the value of the data generated are spatiotemporally constrained (i.e. the pattern might not be observed in a different context). Conclusions drawn are specific within the tested scale and environment, particular population genetic makeup and the interaction between these factors up to generation-specific epigenetic effects. Even so, extrapolation to other systems is a necessary part of any scientific endeavour and is encouraged. Because of this it is important to abstract the observations to broader ecological concepts. Quantitatively results are bound to differ, but qualitatively they may be similar. Such hypotheses should always be interpreted for what they are- hypotheses needing additional research for verification with the same context, and validation within different contexts.

4 Identifying and testing specific stage of development

Ontogeny (i.e. the development of an organism from its earliest stages through maturity (adult)), is particularly crucial in insect farming, such as that of black soldier flies, for understanding how behaviour changes throughout the life cycle and the role that ontogeny plays in shaping adult behaviour.

Black soldier fly development undergoes distinct life stages — egg, larva, pupa, and adult — each with unique behavioural patterns. Within the larval stages, which show voracious feeding behaviour, each larval instar differs in its feeding patterns and nutrient requirements. Larvae focus primarily on feeding, growth, and nutrient deposition, while adults concentrate on reproduction. Understanding the behavioural needs and environmental requirements at each stage is essential for optimising not only growth and resource-use but also the adult stage fecundity. For example, larvae require specific conditions in terms of temperature and humidity, and a third factor, i.e. density, can result in different outcomes for the adult individual. A higher larval density may be preferred for lower feeding time; however, there is a trade-off between development rate and adult size. An individual may develop rapidly but with reduced size or grow larger at the cost of longer development time (Harvey and Strand 2002). Further, density effects are not independent from generally important nutritional parameters, and both impact adult size and fecundity (e.g., Barragan-Fonseca *et al.*, 2018; Gobbi *et al.*, 2013; Jones and Tomberlin, 2020; Zhang *et al.*, 2024). Different life stages of the black soldier fly display varying levels of sensitivity and adaptability to environmental factors like temperature, humidity, and light and biological factors such as conspecific density (Chia *et al.*, 2018; Giannetti *et al.*, 2022; Liu *et al.*, 2020). Understanding how these sensitivities change over the insect's life cycle helps in creating tailored farming environments that maximise survival and productivity at each stage.

Studying ontogeny in the black soldier fly within an industrial setting enables a comprehensive understanding of how insect behaviour evolves over time, providing insights into better farm management, increased efficiency, and higher productivity, and possibly including tailored breeding objectives. By aligning farming practices with the insect's developmental needs, farmers can optimise the full lifecycle of the black soldier fly for sustainable farming operations.

5 Complexity

As discussed in previous sections, complexity is always a topic of discussion with behaviour. Variables, seen and unseen, impact responses of an organism being studied. Furthermore, as pointed out previously, responses to such factors are heavily dependent on the sex, life stage, biological age, and experiences (e.g., associative learning). Therefore, when designing an experiment, it is essential to identify all factors a priori that could impact the tested behavioural response and determining if they can be regulated. If so, an experiment can be developed that is controlled. Then, the measured outputs (i.e. behavioural response) can be linked to the implemented treatments. Conversely, for those that cannot be controlled, they can be addressed in the discussion of the study, which could lead to new studies being proposed, while also recognising current study limitations.

Sex

Sex of an animal dictates in part the responses exhibited. In a general sense, understanding 'motive' of an individual based on sex is key for determining the implications of data generated towards the associated life-history. In the case of insects, such as the black soldier fly, adult males only need to secure a mate, wheras females need to additionally locate, evaluate, and utilise (or not) an oviposition site. It should be noted that similar decisions are also being made with regards to mate choice (i.e., potential partners can be rejected as well). General patterns can be inferred from observing adult flies during maturation, mating and later oviposition (Chiabotto *et al.*, 2024; Laksanawimol *et al.*, 2023); whereby co-factors like size (Jones and Tomberlin, 2020) can be assessed, as well as morphological, physiological and genetic characteristics.

Take for instance the finding that successful mating increases female fitness and lifespan, presumably through nuptial gifts (Harjoko *et al.*, 2023; Manas *et al.*, 2024). Or, that the presence of many competitors not only increases sperm production in males (Manas *et al.*, 2023) but may also motivate females to mate multiple times (Permana *et al.*, 2020, Muraro *et al.*, 2024), as also evidenced through paternity testing (Hoffmann *et al.*, 2021). This may further point to a strategy (e.g., inflated stocking densities) to prevent inbreeding-mediated fitness losses upon sibling mating (Laudani *et al.*, 2024), although this seemingly contrasts genetic data indicating positive assortative mating rather than outbreeding (Hoffmann *et al.*, 2021; Kaya *et al.*, 2021) is at play, at least when interbreeding genetically strongly differentiated populations. However, sex-specific gene expression profiles in a lekking context (Xu *et al.*, 2020) may be strongly associated with female stimuli to males (Rebora *et al.*, 2024), male-specific behavioural sequences like wing fanning as key triggers for female choice (Giunti *et al.*, 2018), and pheromonal cues. Behaviour exhibited can be individualistic or influenced by the presence of other conspecifics. Therefore, when designing an experiment associated with monitoring behaviours, it is critical to determine if the response is sex specific and how such responses might change in the presence of multiple individuals.

Intraspecific interactions

Intraspecific complexity refers to the diversity within a species, encompassing differences in genetics, including variations between sexes, behaviour, and responses to environmental factors. This complexity is key to understanding and optimising insect behaviour in farming, particularly in black soldier fly farming. On the one hand, genetic variation within and especially between adult black soldier fly populations can impact crucial traits like lifespan, reproductive output, and adaptability to environmental factors such as light, temperature, and humidity; while, on the other hand, intrinsic factors such as age, size and sex may also play an equally critical role; for example, age and size influence thermal preference of adult flies (Addeo *et al.*, 2022; Malawey *et al.*, 2021), different sex ratios may influence fertility (Hoc *et al.*, 2019, Putra and Safa'at, 2020), and ageing can affect reproductive choices via interaction with egg load, e.g., laying of unfertilised eggs (Dickerson *et al.*, 2024).

In industrial production, where maximising yield is a priority, understanding this natural variation and its limits is essential. At the same time, genetic diversity also provides an opportunity to selectively breed strains that are more efficient at mating under poor (energy-saving) light conditions, processing suboptimal substrates or that exhibit greater resilience to biotic and abiotic stressors.

Another important facet of intraspecific complexity arises from group dynamics. While black soldier fly larvae must balance competition for resources with collective feeding behaviour, the adult black soldier flies are also expected to be significantly influenced by population density. With males often competing for access to females, and females vying for optimal oviposition sites, conspecific density may affect behaviour by influencing reproductive output.

Interspecific interactions

Interspecific interactions refer to relationships between different species. In controlled laboratory settings or indoor mass-rearing facilities, where a single insect species is typically reared in isolation. Such interactions are minimised by strict protocols that prevent contact with external species and avoid contamination risks. However, in natural environments, interspecific interactions are common and can significantly influence insect behaviour and survival. For example, black soldier fly larvae compete for resources with house flies, *Musca domestica*, L. (Diptera: Muscidae). While early studies suggested that black soldier fly larvae could serve as natural control agents for house flies, recent research points out the importance of colonisation sequence: the presence of house flies early in the process can deter black soldier fly colonisation, affecting their survival and reproductive success (Furman *et al.*, 1959; Miranda *et al.*, 2019). Likewise, parasitoids can be problematic, and for the black soldier fly occur in

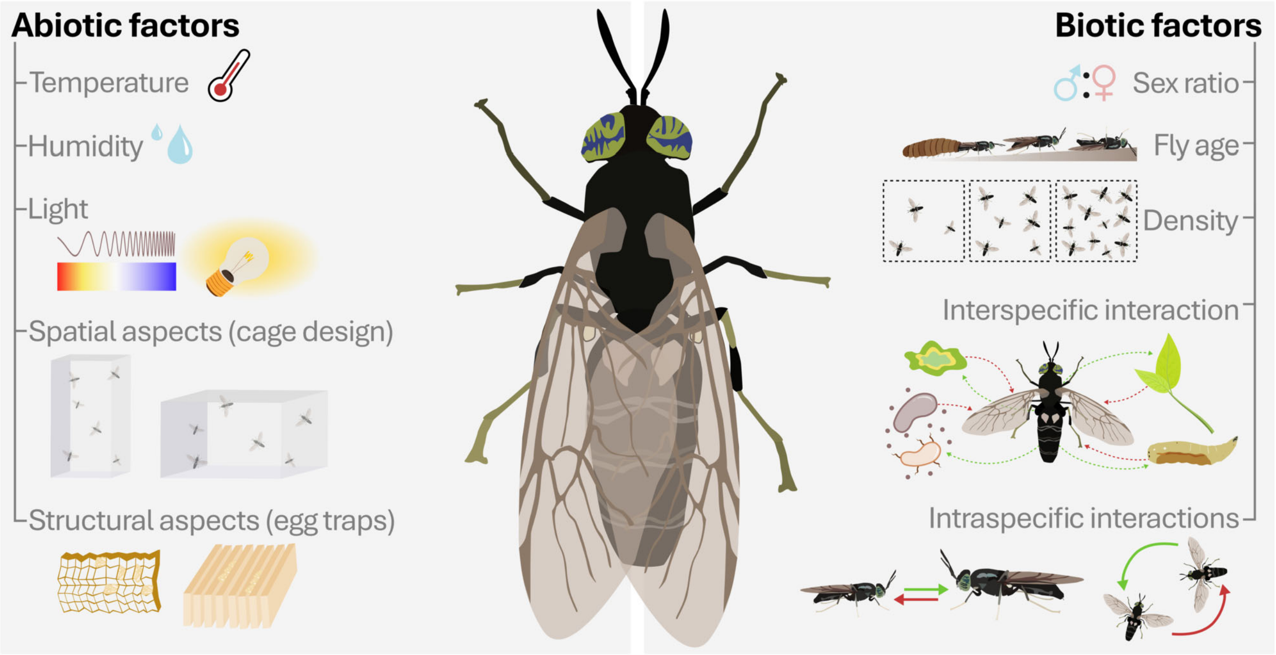

FIGURE 1 Abiotic and biotic factors influencing insect behaviour.

some global regions like West Africa (Ewusie *et al.*, 2018; Maquart *et al.*, 2020) or Asia (Binoy *et al.*, 2023). However, interactions with microorganisms (primarily bacteria and fungi) are unavoidable in rearing systems (axenic larvae exempted) and their relevance should not be underestimated (Auger *et al.*, 2023; Wynants *et al.*, 2019). For black soldier fly larvae, interdependencies with microbial life have been extensively studied (see Auger *et al.*, 2025), as larval digestion relies on microbial support and microbial activity is a promising setscrew for enhancing conversion performance (Gold *et al.*, 2018). The role of microorganisms in the digestion of adult stages of the black soldier fly has received little attention so far, as it has long been assumed that adult flies do not feed, which is not necessarily true if given access to food (Bertinetti *et al.*, 2019). Interactions with microbes influencing adult behaviour, either on conspecific eggs or inherently attractive oviposition substrates (which also represent suitable feed resources for the fly's offspring), have attracted greater interest due to the potential to gain better control and efficiency in a key bottleneck – the maximisation of viable offspring (Kortsmit *et al.*, 2023a; Pastor *et al.*, 2015; Zheng *et al.*, 2013). This research is working towards enhancing our understanding of semiochemical communication between microbes and flies as a means for interspecific interaction (Davis *et al.*, 2013).

Abiotic conditions

Adult flies are sensitive to a multitude of abiotic environmental factors (Figure 1) that define their reproductive success, and mimicking quasi-natural conditions in a replicable way in artificial settings is a key challenge. Temperature, humidity, and light are the most fundamental abiotic parameters for optimising mating and oviposition (Tomberlin and Sheppard, 2002). These factors need to be adapted to the developmental stage and behaviour of insects (see Coudron *et al.*, 2025). Choosing the right light source in terms of intensity (Schneider 2020), wavelength (Oonincx *et al.*, 2016), colour (Klüber *et al.*, 2020), and photoperiod (Hoc *et al.*, 2019) has significant effects on mating and reproduction. For example, for the black soldier fly, the majority of mating pairs (75%) took place at a light intensity greater than 200 μmolC/m^2 per s but none occurred at a light intensity >63 μmol/m^2 per s (Tomberlin and Sheppard, 2002).

In addition, spatial and structural aspects align with the behaviour of insects, such as appropriate cage design and structures for oviposition, to guide females to deposit their eggs in a specific, designated location (Lemke *et al.*, 2024a). For example, large cages can feature ventilation systems designed to promote optimal reproductive behaviours, such as by maintaining humid air concentrated with attractive volatile organic compounds in areas near egg traps (Salari and De Goede 2023, 2024). Furthermore, egg traps can be constructed using various materials, including corrugated cardboard (Choi *et al.*, 2016; Raman *et al.*, 2024), wood (Julita *et* *al.*, 2021; Raman *et al.*, 2024), floral foam (Choi *et al.*, 2016), and plastic (Raman *et al.*, 2024). Crevices of 1 mm have been found to be the most suitable for black soldier fly oviposition (Bogdan *et al.*, 2022). However, no single material has been identified as universally optimal, as each material offers distinct advantages and limitations. The selection of materials should be tailored to specific experimental needs, such as durability, ease of handling, suitability for the insect species, and the environmental conditions being studied.

6 Methods for conducting behaviour research

Early research on reproductive behaviour of the black soldier fly (Tomberlin and Sheppard, 2001, 2002) describes adults as engaging in aerial swarms and lekking. Lekking is a multi-component behaviour in which males emerge prior to females due to protandry, and establish a mating site distinct from any resource, where they then jostle for an advantageous position (Alcock, 1987, 1990). Females then visit the lek strictly to mate, before leaving to oviposit (Alcock, 1987). For wild black soldier flies, the distance between their lekking site and the oviposition site can be several hundred (Tomberlin and Sheppard, 2001) to several thousand meters apart (Lemke *et al.*, 2023) or more. As such, there have been disagreements as to whether lekking persists within the confines of cages either in whole (Lemke *et al.*, 2023) or in part (Lemke *et al.*, 2024a) since available space is reduced several-fold.

Of course, for industry, the goal is to increase total production of fertile eggs, for minimal cost/inputs (Boller, 1972). As such, behavioural experiments have typically focused on the number of clutches per female and eggs per clutch, the total yield (per cage or per fly) and hatch rate, as well as on mating- and oviposition-counts. These are measured in response to varying biotic (e.g., population density (Hoc *et al.*, 2019), sex ratio (Hoc *et al.*, 2019; Putra and Safa'at 2020), size-classes (Gobbi *et al.*, 2013, Jones and Tomberlin, 2020), demography (Addeo *et al.*, 2022; Dickerson *et al.*, 2024) or abiotic conditions (i.e., lighting (Lemke *et al.*, 2024b; Liu *et al.*, 2020; Zhang *et al.*, 2010), temperature (Addeo *et al.*, 2022), humidity (Tomberlin and Sheppard, 2002), surface area (Lemke *et al.*, 2024a), cage or egg-trap material (Julita *et al.*, 2021), availability of adult nutrition (Bertinetti *et al.*, 2019; Klüber *et al.*, 2023; Lupi *et al.*, 2019)).

However, at full-scale, some of these metrics can be difficult to ascertain (especially mating counts) since there can be as many as 20 000 flies/m^3. As such, simulations have been developed to connect patterns across spatial scales (Kobelski *et al.*, 2024). Additionally, since there can sometimes be a disconnect between these factors temporally (i.e. more mating does not necessarily lead to higher egg production or fertility) (Dickerson *et al.*, 2024), factors that precede oviposition within the 'chain' of reproductive events (Lemke *et al.*, 2023) can be studied to understand their downstream impact on fertility.

Accordingly, the goal of behavioural research has expanded towards documenting relevant individual behaviours (Serge *et al.*, 2023), while also understanding the role of intraspecific competition (Chiabotto *et al.*, 2024), mate choice (Giunti *et al.*, 2018), communication and sexual signals (Rebora *et al.*, 2024), the mating system (Hoffmann *et al.*, 2021; Muraro *et al.*, 2024), as well as sexual conflict (Manas *et al.*, 2023, 2024, Munsch-Masset *et al.*, 2023). Simultaneous to such effort, the development of ethograms and verbal models of field-caught populations can contextualise findings of captive flies via comparative studies (Masse *et al.*, 2022).

Wild behaviour

Studying the black soldier flies in their natural habitat provides baseline data on their natural behaviours and environmental interactions. This helps understand their ecological roles and the conditions that drive behaviours like mate selection, lekking, or oviposition site selection (Addo *et al.*, 2024; Ewusie *et al.*, 2019; Ferdousi *et al.*, 2024; Santos *et al.*, 2022). However, controlling variables in the wild is challenging, and environmental factors such as actual weather, as well as maternal imprinting (Queller, 2003), can introduce significant variability.

Cage or lab-based testing

In controlled environments, researchers can manipulate specific variables (e.g., temperature, humidity, light) to study black soldier fly behaviour in detail. Lab testing also allows for the randomisation and replication of experiments, providing more consistent and reliable data. However, caged behaviour might differ from wild behaviour due to the constraints of confinement.

Field-to-lab-to-industrial scale

While moving from field to lab experiments allows for controlled conditions and incorporations of technical tools like sensors and imaging devices; in industrial settings, behaviour is often tested on a larger scale. Factors like industrial cage design including aspect ratio,

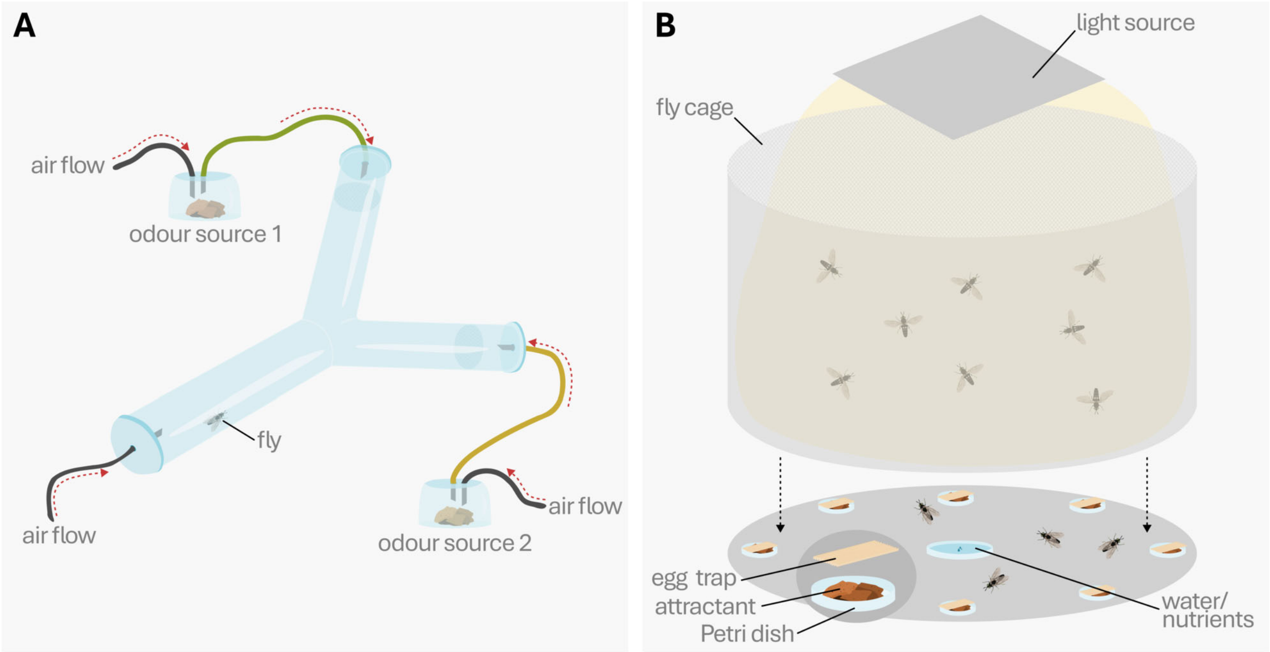

FIGURE 2 Set-up of two types of choice test: (A) Y-tube test and (B) ‘cafeteria assay’.

population density, space, and resource availability may drastically alter black soldier fly behaviour, which is why such extrapolation at different scales needs careful calibration.

Wind tunnel and Y-tube test

Examining adult insect behaviour in the lab presents numerous challenges as data collected often provide insights to responses in artificial environments. Furthermore, the devices utilised to explore such behaviours indoors are often restrictive to smaller scales (e.g., Y-tube discussed below). However, some equipment does provide larger scale response opportunities. Wind tunnels are an example of a device that allows for testing various abiotic and biotic factors regulating insect behaviour. Biotic factors, such as the presence of multiple individuals (Ahmed and Faruque, 2022), sexes (Kainoh, 2011), ages (Parra *et al.*, 1996), or resources (e.g., pheromones; Knudsen *et al.*, 2018) can be explored in conjunction with abiotic factors, such as wind speed (Aluja *et al.*, 1993) and dynamics, light abundance (Gu and Dorn, 2001), temperature, and humidity (Knudsen *et al.*, 2018; Miller and Roelofs, 1978). While these tunnels can range in size from 0.5 to several meters in length and diameter, they are restrictive as they are still enclosed. Consequently, translating data from such environments to the real world can be limiting.

A Y-tube (Figure 2A) is used to conduct behavioural assays to study insect preferences by challenging the insect to choose between two pathways. Each pathway is associated with a different stimulus (e.g., a specific resource or chemical cue) (Geier and Boeckh, 1999). With this assay, the insect’s response to certain stimuli can be tested under controlled conditions, however, this also represents a key limitation as it is not possible to replicate natural conditions, and various confounding variables might influence its choice. In black soldier fly research, Y-olfactometry has been successfully used to investigate the effect of fatty acids in attracting females to oviposition sites (Klüber *et al.*, 2024).

Choice test

Similar to the Y-tube, a choice test (or ‘cafeteria assay’) (Figure 2B) offers a broader way to test an animal’s preference for a specific resource (e.g., feed source, mate, oviposition site) and is frequently used for insect studies (Kinzner *et al.*, 2016; Lihoreau *et al.*, 2016; Martel and Boivin, 2011). In this test, potentially attractive substrates are set-up in circular order to guarantee equidistance from another and equipped with appropriate egg traps. The flies are set free in the centre of the cage and left to choose from the provided substrates. The most attractive cues emitted by the substrates will guide the fly to the respective egg trap. The number of simultaneously presented choices affects the discernability of behavioural differences in a non-linear way and factors including insect species, substrates, physiology, type of stimulus determine the ideal number of choices (Raffa *et al.*, 2002). Adequate replication should be ensured to obtain sufficient statistical power and may be implemented; (1) at the substrate level, by providing multiple replicates of each substrate within each cage in randomised order and (2) at the cage level, by using multiple cages with the same substrates but in different order within each cage. This way, both neighbourhood effects of substrates (how a substrate affects the attractiveness of its neighbouring substrates) and cage effects (how microclimatic conditions in a climate room affect experimental outcomes) can be taken into account. While three replicates represent the absolute minimum for statistical evaluation, higher replication is recommended and often required to obtain meaningful results in behavioural studies, which typically entail high variability. However, the feasible replication level also depends on space for the experimental setup (e.g., climate cabinet vs. climate room), and available personnel to manage the workload associated with high replication within an acceptable time frame (e.g., sampling, observations). Randomisation of spatial configuration is crucial, as independence among presented substrates cannot be assumed and configuration effects can affect comparability among similar studies (Raffa *et al.*, 2002). To illuminate the underlaying mechanism of preference, the choice test aims to ultimately identify the true choice of an insect (as opposed to the apparent choice), by fulfilling that; (1) the choice is not random, (2) the choice is consistent regardless of any influence by the provided resource, and (3) each resource is perceivable by the insect to avoid sensory bias (Martel and Boivin, 2011).

The design of the cage in which the test is conducted should support the fly's natural reproductive behaviour to reduce the risk of confounding factors influencing the choice of resources, as specifically cage dimensions can affect reproductive success (Giunti *et al.*, 2018; Lemke *et al.*, 2023). In addition, fly density based on cage volume and sex ratio need to be considered for optimal results. For the black soldier fly, a density of 0.0065 flies/cm^3 and a slight female bias has been demonstrated to enhance the yield of fertilised eggs and hatching rates (Hoc *et al.*, 2019). Putra and Safa'at (2020) found that a male-to-female ratio of 40:60 led to higher egg production compared to a 60:40 ratio or equal distribution of males and females. The environmental conditions within the cage should be adapted to species-specific requirements by providing a suitable light source, day-night rhythm, ambient temperature, and humidity (Hoc *et al.*, 2019). Since cages are largely self-contained, environmental conditions inside can deviate from those in the climate chamber in which the experiment is conducted, particularly if air exchange is impaired by inadequate ventilation or the materials used in cage construction.

Disturbances during the choice test should be reduced to a minimum as opening the cage can cause flies to escape or get killed while interfering with the experimental set-up. Variation in technique and environmental interaction affects the reliability and reproducibility of the experiment by introducing sampling bias, making it difficult to isolate true biological signals (Biro, 2013). However, depending on the duration of the experiment, replacing the egg traps may be necessary to avoid the hatching of larvae or replenishing water and/or food sources within the cages. Key parameters to assess during a choice test investigating oviposition preferences include egg biomass, oviposition timing, longevity of flies, and differences in survival among sexes. By regularly observing or video-graphically documenting fly behaviour within the cages, additional conclusions about preferences that did not lead to oviposition can be made. During the disassembling of the experimental set-up, attention should be paid to misplaced egg clutches that can either be relevant for the data or serve as inspiration to improve the cage design for further studies.

Electrophysiology

Electrophysiological analyses focus on recording electrical activity within the nervous system of the insect. Once substrates eliciting a sought-after response have been determined, electroantennography can be used to qualitatively measure a fly's reaction to single components, such as a complex smell. This method provides information on the changes in the electrical potential induced by olfactory sensory neurons within an antenna after exposure to specific stimuli (Olsson and Hansson, 2013). To dissect complex smells and purify their components, electroantennography is typically coupled with gas chromatography (Struble and Arn, 1984). A more refined, quantitative picture of an insect's reaction to stimuli can be achieved by single sensillum recording, which measures electrical activity in single sensilla (sensory organs of arthropods containing neurons) and can also be combined with gas chromatography (Olsson and Hansson, 2013). Using this extracellular technique, neuronal activity is correlated with established odour–response patterns (Lin and Potter, 2015). Multielectrode recording can simultaneously target multiple neurons, thus providing indications for neural circuits and processing sensory stimulation (Bhavsar *et al.*, 2016). Correctly placing the multielectrodes in small insects represents a technical challenge, limiting the number of neurons that can be simultaneously recorded, and adaptations to different sensory systems are required. The electrodes should be precisely placed in targeted neuronal compartments or sensory processing regions (e.g., for olfaction, vision, mechanosensation, or auditory processing) and their correct placement should be verified after recordings, as incorrect placement can result in high signal-to-noise ratios, movement artifacts or unreliable recordings (Bhavsar *et al.*, 2016).

Data from electrophysiological analyses should be verified using behavioural assays (e.g., real-time observations of mating or oviposition preference in controlled environments using Y-tube or choice tests). Doing so will establish meaningful links between sensory input and behavioural output. Insects often use a combination of olfactory, gustatory, visual, and tactile cues to choose an oviposition site, further highlighting the need for behavioural assays (Martel and Boivin, 2011). Moreover, the transferability of observations in nature, where a variety of resources are available to choose from, to artificial settings where defined and controlled diets are required for stable production, the induction of natal habitat preference may be a limitation (Davis and Stamps, 2004).

Neuron level responses

Environmental stimuli such as light, taste, or smell introduced into experimental settings need to be controlled to assess their effects on insect behaviour. In black soldier fly adults, vision plays a crucial role; they possess three simple eyes and two compound eyes sensitive to blue, green, and ultraviolet light (Oonincx *et al.*, 2016). Their photoreceptors show peak sensitivity at wavelengths of 440 and 550 nm, aligning with blue and green light (Schneider, 2020). Proper light sources are essential, as the absence of key wavelengths has been shown to impede mating behaviour (Zhang *et al.*, 2010).

In addition to visual stimuli, chemosensory mechanisms also define how black soldier flies interact with their environment. Investigations on the molecular basis of chemoreception have shown that, compared to other dipteran species, the black soldier fly possesses a reduced number of gustatory receptors which are responsible for taste and contact perception (Xu *et al.*, 2020). However, genes related to chemoreception are among the most expanded gene families in the black soldier fly. A genomic study revealed 153 genes encoding for olfactory receptors – twice as many as in the house fly – highlighting the importance of smell in their behaviour (Zhan *et al.*, 2020).

Odorant binding proteins are crucial for detecting volatile organic compounds, and their specificity can be predicted *in silico* (Xu *et al.*, 2020). The expression of these proteins varies across developmental stages, with only about half of the chemoreception proteins shared between adults and larvae, pointing out the different chemosensory requirements of the black soldier fly during its life cycle (Scieuzo *et al.*, 2021). Moreover, male and female adults show morphological differences in their antennae, and some odorant receptors are only expressed in female flies and are thought to be involved in detecting signals related to oviposition (Pezzi *et al.*, 2017, Xu *et al.*, 2020). Volatile organic compounds that bind specifically to female-expressed odorant receptors may serve as indicators for suitable resources for their offspring, making them promising candidates for promoting oviposition behaviour in controlled settings (Scieuzo *et al.*, 2021).

7 Outcomes

Measuring behaviour of an adult or immature insect can lead to crucial discoveries with applications with the insects as food and feed sector. As discussed throughout this chapter, clearly defining the methods employed is critical as each approach results in specific outcomes. In fact, these outcomes can be relevant to the industry (e.g., increased fertile egg production, optimised environmental criteria for waste conversion) or to the broader community as related to ecology, evolution, and conservation. However, as mentioned in the introduction, this chapter utilized the black soldier fly as a model; therefore, caution is urged when applying such information to other insect models.

For this chapter, the key outcomes are aimed at the mass production of insects where efforts are strictly focused on optimising production while minimising costs. With recent developments in animal welfare, such efforts could lead to better conditions for housing massive numbers of insects while minimising their stress. Thus, the following section is focused on industry and conservation values. The goal is to demonstrate a positive return on investment when exploring insect behaviour as related to industrialisation.

The industrialisation of insect farming requires scaling up to larger operations, which brings both challenges and opportunities for studying and managing insect behaviour (Athanassiou *et al.*, 2024; Meneguz *et al.*, 2023; Pastor *et al.*, 2015). As production transitions from lab settings to small-scale experimental farms and eventually to large-scale commercial operations, effectively understanding and managing insect behaviour becomes essential to maintaining efficiency, productivity, and consistency.

As discussed earlier, larger populations in confined spaces can result in complex group dynamics that are not apparent in smaller or lab-scale operations. Issues like overcrowding, egg deposition in deceased flies, and haphazard egg laying can affect productivity. Moreover, maintaining optimal conditions — temperature, humidity, ventilation, and lighting — across vast breeding units may become more challenging as operations grow. Adult black soldier fly behaviour is not only sensitive to environmental fluctuations but also affects its surroundings across space (i.e. scale) and time (i.e. generation), and an extrapolation-based scaling up may increase the risk of suboptimal conditions, which can negatively affect productivity. It is therefore critical to investigate and understand the response of adult black soldier flies to such factors that become crucial on an industrial scale.

Large-scale black soldier fly farms often use automated systems for increased efficiency in feeding, rearing and environmental control. Optimising these systems requires a deep understanding of black soldier fly behaviour. For instance, knowing peak hatching times of adults and ideal environmental conditions can help design smarter, consistent, and more efficient automation for breeding and rearing. Additionally, in large-scale farming, insects are more susceptible to stress and disease due to higher population densities and microbiological factors (Vogel *et al.*, 2022). Whereas impaired larval growth due to dietary heavy metals, mycotoxins or pesticides (Heuel *et al.*, 2023; Meijer *et al.*, 2024; Purschke *et al.*, 2017) presumably confers direct adult fitness reduction (Jones and Tomberlin, 2020), less tangible pathogen-mediated premature immune responses likely still involve such trade-offs in the adult stage (Shah *et al.*, 2024). Understanding the behavioural impacts of stress within and across developmental stages, or even trans-generational effects (Mukherjee and Vilcinskas, 2019), such as reduced reproductive success, including causal mechanisms is crucial for mitigating these risks and ensuring consistent production output.

Aligning basic research and applied perspectives within mutually beneficial approaches will progress in the insect farming sector. For instance, positive assortative mating (Hoffmann *et al.*, 2021, Kaya *et al.*, 2021) seems to contrast indication for polygynandry across strains (Muraro *et al.*, 2024; Permana *et al.*, 2020). Possibly, such behaviour serves for female fitness maximization via exploiting male nuptial gifts (Harjoko *et al.*, 2023; Manas *et al.*, 2024), but is complex given post-mating sperm competition, as indicated by male spermatogenesis and morphology of the female reproductive tract (Manas *et al.*, 2023; Malawey *et al.*, 2019; Munsch-Masset *et al.*, 2023). These traits of the mating system, coupled with recent evidence for male sperm plugs to maximize their fitness in turn (Manas *et al.*, 2024), suggest that the causes and consequences of black soldier fly behaviour are complex. Possible sexual conflicts that are likely relevant in a production context appear to be deeply rooted in an evolutionary ecology context. Importantly, existing frameworks, such as the seminal research in the Dipteran model *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) regarding reproductive behaviour (Anholt *et al.*, 2020) as well as neuromodular regulation of feeding (Pool and Scott; 2014), can not only be built on, but also be broadened by the global black soldier fly community.

From an applied perspective, these behaviours form the foundation of sustainable insect farming systems, transforming organic waste into valuable biomass at industrial scale. The challenges and opportunities of scaling in both experimental approaches and production contexts are addressed from various angles (Coudron *et al.*, 2025; Deruytter *et al.*, 2025). However, not every stage of the insect's life cycle and every behaviour of a developmental stage may respond equally well to a given extent of scaling. For example, neonates are so small that scaling their rearing even by a few 10-folds may create issues with heat loss thus limiting the level of scaling and leading to reduced efficiency beyond it (pers. observations). Similarly, overly large scales may result in uneven distribution of larvae and feed, causing inconsistent growth and output. The scale of the scaling-up, therefore, needs to be aligned with the learnings from research into insect behaviour. As the world increasingly looks for sustainable and efficient solutions to global challenges, black soldier fly behaviour research holds immense promise for addressing issues like food security, waste management, and resource efficiency.

The extent to which adult behavioural phenotypic variation exists among global wild populations remains to be investigated yet given the marked genetic structure of native and non-native black soldier fly (Kaya *et al.*, 2021) some degree of adaptation to different environmental contexts, such as different degrees of seasonality or solar courses, would not be surprising. Nevertheless, when transferring a population from the wild to captivity, the genetic makeup of the source population is merely equally crucial than genetic fate of the founder subpopulation. Key aspects like population establishment, managing genetic diversity and monitoring selective breeding are detailed in Sandrock *et al.* (2025). Yet, it is important to highlight that a change of setting, which prevents exhibiting a certain behaviour, or renders parts of a harmonised courtship cascade useless in a specific environment (e.g., as a consequence of limited space, poor light, insufficient humidity, inadequate pheromonal cues etc.) will instantaneously impact the proportion of individuals successfully reproducing and contributing to the next generation, and consequently genetic profiles of all future generations. Insofar, regardless of finally targeted scale of rearing, what counts is effective population size (Espinoza, 2022), with single severe population genetic bottlenecks readily leaving signals that can be traced even many generations later (Kaya *et al.*, 2021). Next to possibly limited initial genetic variation due to field collection constraints (Ewusie *et al.*, 2019), it has been demonstrated that population genetic diversity can quickly erode due to insufficient adaptation to artificial conditions resulting low effective population size possibly followed by an inbreeding vortex (Rhode *et al.*, 2020). Although selection for black soldier fly larval traits was shown to impact population genetic variation during early domestication (Hull *et al.*, 2023, 2024), moderate inbreeding per se seems unproblematic or even beneficial for certain production parameters (Cai *et al.*, 2022). This points at adult traits playing an essential role for successful population establishment. Indeed, worldwide most widely distributed black soldier fly strains going back to a decisive North American founder population (Sheppard *et al.*, 2002) exhibit pronounced genome-wide signatures characteristic for domestication (Kaya *et al.*, 2021, Generalovic *et al.*, 2023). Inspection of genome-wide selective sweeps suggests several key adult traits have been targets to mass rearing selection during domestication of this origin, which is further supported by partly convergent patterns in more recently managed populations from genetically distant wild populations (Generalovic *et al.*, 2023). Highlighted candidate genes included putative roles in adult brain function, sensory perception and stimulus response, male-male aggression and sperm function, circadian rhythm regulation and photoreceptor differentiation, glycogen metabolism and susceptibility to obesity, or cuticle proteins and immune function (Generalovic *et al.*, 2023). Understanding relevant adult traits, their genetic architecture and putative genome-wide effects of domestication will facilitate to harness the breeding potential across the entire black soldier fly life cycle.

A prerequisite for selecting adult traits is adequate phenotyping. This includes some larval traits, since increased and/or more uniform growth rates as well as possible trade-offs regarding protein versus lipid contents are relevant for adult fitness (Boggs and Freeman, 2005). Although progress is being made towards non-invasive assessments, including immature sexing (Nawoya *et al.*, 2024), exploring adult behaviour (Laursen *et al.*, 2021) and environment-dependent gene expression (Malawey *et al.*, 2021; Xu *et al.*, 2020), as well as controlling mating, e.g., in pedigree-based breeding schemes (Jensen *et al.*, 2024), there are still challenges to be tackled (Nawoya *et al.*, 2025). Importantly, adult behavioural traits during reproduction becoming selection targets themselves require careful scaling and parameter settings in the nucleus breeding population compared to large-scale production, as well as dedicated phenotyping records allowing undisturbed intraspecific interactions and individual tracking until oviposition and death (taking advantage that adults do not molt anymore).

Apart from that, the sector needs to be aware of two additional aspects relevant in a behavioural context. First, selective breeding that potentially affects reproductive behaviour in a production context optimally entails that insect welfare also be considered proactively (Barrett *et al.*, 2023). In this sense, diet likely affects immature and later adult behaviour (Nayak and Mishra, 2021) and should be carefully chosen, and wingless flies (Zhan *et al.*, 2020) might be a breeding objective in order to save space, but is arguable (Lemke *et al.*, 2023). Increased male-male aggression as a consequence of otherwise reduced resource competition and crowding, as found for crickets (Olzer *et al.*, 2019), however, indicates the risk of unintended side-effects on complex behaviours be monitored carefully. A profound knowledge on the biology of farmed insects, necessarily including behaviour and cognition, is thus key to settle adequate welfare concepts in insect production (Kortsmit *et al.*, 2023b; Voulgari-Kokota *et al.*, 2023).

Second, adult 'stubbornness' is probably the major reason why global managed populations of the black soldier fly barely capture genetic diversity of local wild black soldier fly populations, but mostly domesticated strains pre-adapted to farming contexts. These latter, if not well confined, however, can mediate gene flow into local wild populations (Generalovic *et al.*, 2023; Kaya *et al.*, 2021). The progressing genetic assimilation that results could deprive unique natural genetic resources from future breeding initiatives, which could become a veritable conservation genetics issue particularly in the indigenous ranges of the Americas (Kaya *et al.*, 2021; Ståhls *et al.*, 2020). Moreover, genetic admixture has the potential to alter ecological responses in insects and facilitate invasiveness (Bang and Courchamp, 2021, San Jose *et al.*, 2023). Even the best lobby advertisement for sustainable food chains would struggle to restore the reputation of a pest insect.

8 Synthesis

While this article focusses on the black soldier fly as a model for discussing behaviour research, each topic reviewed applies to other production insects analogously. Furthermore, this chapter only provides a snapshot of selected critical factors, and it should be noted that influences on insect behaviour are presumably numerous and strongly interactive. In this sense, the value of corresponding research efforts cannot be understated. Yes, such data provide greater insights as to optimising production; yes, such efforts need to be applied within all entities as results will likely be unique to their population in their setting, and yes, such efforts can result in increased returns on invest and improved ecological sustainability alike. However, the value of such work is not limited to applied outcomes. Research on insects produced for food and feed can also serve as a platform that bridges with the basic sciences. Such partnerships can lead to discoveries that have greater impacts beyond insects as food and feed.

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Conflict of interest

No conflicts of interest are known.

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