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Welfare considerations for farming black soldier flies, *Hermetia illucens* (Diptera: Stratiomyidae): a model for the insects as food and feed industry

M. Barrett[1,2*], S.Y. Chia[3], B. Fischer[2,4] and J.K. Tomberlin[5]

[1]Department of Biology, California State University Dominguez Hills, Carson, CA 90747, USA; [2]Rethink Priorities, 530 Divisadero St, San Francisco, CA 94117, USA; [3]Laboratory of Entomology, Wageningen University and Research, P.O. Box 16, 6700 AA Wageningen, the Netherlands; [4]Department of Philosophy, Texas State University, 601 University Dr, San Marcos, TX 78666, USA; [5]Department of Entomology, Texas A and M University, 2475 TAMU, College Station, TX 77843, USA; meghan.barrett21@gmail.com

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REVIEW ARTICLE

Abstract

Over two hundred billion black soldier flies (BSF, *Hermetia illucens* (Diptera: Stratiomyidae)) are reared annually across the globe, with the industry projected to grow substantially in the coming decade. Black soldier flies are being actively explored across the globe for use as livestock feed; fishmeal replacements; biodiesel; human, animal, and food waste management; and even sustainable human protein. Despite the huge number of individual insects reared and interest in BSF welfare by numerous producers and academics, there is no paper that considers the species-specific welfare of BSF in farmed conditions. We review factors that relate to BSF welfare in commercial rearing facilities, including: diseases/parasites, abiotic conditions (temperature, humidity/moisture, substrate aeration, light, pupation substrates, and adult spatial needs), adult and larval nutritional considerations, injury and crowding, handling-associated stress, selective breeding and genetic modification, environmental contaminants, and slaughter methods. We conclude with a discussion of the most pressing welfare concerns for the industry, recommendations for altering the conditions that give rise to them, and suggestions for future research directions that would lend valuable insights to BSF welfare. While this summary is BSF-centric, the core topic of animal welfare applies to all insect models currently, or in the future, produced as food and feed.

Keywords: insect welfare, rearing conditions, survival, slaughter methods

1. Introduction

Approximately 200-300 billion individual black soldier flies (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae), are estimated to be farmed annually and the industry is expected to grow (Rowe, 2020). The vast majority of farmed BSF are slaughtered in the larval stage, although adults are also reared as brood stock. BSF larvae are reared for their incredible biomass conversion abilities, including of organic waste (Cicková *et al.*, 2015; Lalander *et al.*, 2015), as well as their favourable nutritional composition for feeding livestock and exotic pets. BSF larvae may also reduce greenhouse gas emissions and noxious volatile organic compounds associated with waste decomposition (Beskin *et al.*, 2018; Pang *et al.*, 2020; Popa and Green, 2012) and are being explored as a source of biodiesel (Lee *et al.*, 2021), a protein source to feed a growing human population (Hopkins *et al.*, 2021), a way to improve waste management (Banks *et al.*, 2013), and a mechanism of economic growth in developing nations (Chia *et al.*, 2019).

Despite the huge number of individual insects reared, and interest in BSF welfare by numerous producers, academics, and consumers (Bear, 2019, 2021; De Goede *et al.*, 2013; Delvendahl *et al.*, 2022; Erens *et al.*, 2012; IPIFF, 2019), species-specific information related to the welfare of farmed BSF is currently lacking. This paper outlines an approach to BSF welfare assessment, BSF biology, current industry rearing practices, and the many variables that may influence BSF welfare during rearing and slaughter, as the first comprehensive, species-specific paper on BSF welfare in the industry. We end with recommendations for welfare improvements, areas of future concern for BSF welfare as the industry continues to grow, and a list of future research directions that are necessary to improve BSF welfare in farmed conditions.

Insect welfare framework

The dominant theory of animal welfare focuses on animals' subjective states: an animal's welfare is how the animal is faring from the animal's point of view. Given this theory, it matters a great deal whether animals have subjective states, and in particular, whether they have positively and negatively valanced subjective states. Animals that have such states are sentient. The question of whether insects are sentient is far from resolved (for discussion, see Adamo, 2016; Barron and Klein, 2016; Birch, 2020; Lambert *et al.,* 2021) and beyond the scope of this paper. Here, we employ a precautionary principle: since insects may be sentient, we should treat them as though they are sentient (Birch, 2017; Mikhalevich and Powell, 2020; Van Huis, 2021).

This approach may be considered excessively cautious (though the precautionary principle is already used by some insect producers to justify their attentiveness to welfare-based rearing practice; Bear, 2021), however there are many moral and practical reasons to be cautious when it comes to insect welfare. First, there is extensive disagreement about both the science of consciousness generally and the science of sentience specifically (for a sense of the range of theories on offer, see Browning and Birch, 2022; Del Pin *et al.,* 2021). Thus, while many people may have strong intuitions that insects are likely not sentient, those intuitions cannot claim the authority of scientific consensus. Second, there are reasons why researchers might not detect sentience in insects even if, in fact, they are sentient. Due to the significant challenges posed by differences in vertebrate/invertebrate anatomy and behaviour, few studies have been designed to test for markers of sentience with much confidence. So, less evidence for or against insect sentience may not indicate much about if they are, actually, sentient. Third, in the period since the Scientific Revolution, the historical trend is toward the view that sentience is more widely distributed in the animal kingdom – not less (Proctor, 2012). So, it would not be surprising if future science were eventually to conclude that insects are sentient. Finally, there are pragmatic reasons for the industry to adopt practices that will not have to be revised later, due to pressure either from the public or regulators. Retooling production facilities and SOPs can be very expensive both monetarily and in public relations terms. It seems prudent to avoid those expenses if possible.

If we do adopt a precautionary approach, we are left with the difficult question of how to assess BSF welfare. One frequent proposal (Erens *et al.,* 2012; IPIFF, 2019; Van Huis, 2021; but see Gjerris *et al.,* 2016), is to extend the five freedoms model to insects. According to that influential model of animals' interests (Brambell, 1965), animals ought to kept in ways that keep them free: (1) from hunger and thirst; (2) from discomfort; (3) from pain, injury, and disease; (4) to express normal behaviour; and (5) from fear and distress. Erens *et al.* (2012)'s proposal is helpful insofar as the five freedoms (now usually understood in terms of the five 'domains') provide specific goals for producers. For insects, though, there is little information available on individual pain, fear, discomfort, or distress; and, for BSF specifically, there is little information available about natural behaviours. As a result, it is challenging to use more granular standard welfare assessment frameworks to do welfare assessments for insects. So, we pursue two strategies to better understand BSF welfare given the current state of knowledge.

First, we use population-level mortality as a very coarse proxy for the subjective welfare of individuals – which, given the assumptions above, is what matters. The advantage of this proxy is that it can be assessed straightforwardly and is clearly a sign of individual stress. And, if BSF are sentient, then it is plausible – though, admittedly, far from certain – that this stress would induce negative subjective states.

Second, we discuss sublethal factors that may lower BSF welfare in industry conditions. Such factors include diseases/parasites, abiotic conditions (temperature, humidity/moisture, substrate aeration, light, pupation substrates, and adult spatial needs), adult and larval nutritional considerations, injury and crowding, handling-associated stress, selective breeding and genetic modification, environmental contaminants, and slaughter methods. Granted, apart from inducing mortality, there is considerable uncertainty about the welfare impacts of each of these factors. In each case, however, similar factors in farmed vertebrate species are associated with negative welfare impacts. So, although much more research is necessary, it seems reasonable to canvas these possible vectors of poor welfare.

Black soldier fly biology

Understanding the biology of BSF is critical to contextualising potential welfare concerns that may arise in industry settings. The BSF is native to the Neotropics but has spread to most of the world due to globalisation. The number of generations per year can be influenced by the seasonal changes in light, temperature, and/or humidity of the region (e.g. Park *et al.,* 2010); in the tropics, BSF can breed year-round.

Larvae go through six stages (Kim *et al.,* 2010), with the final post-feeding 'wandering' stage featuring increased sclerotisation and the darkening of the cuticle from cream to brown by the time of pupariation. Larvae are photophobic and bury themselves in either their feeding substrate or a drier pupation substrate. Larval development time and growth are strongly influenced by diet and abiotic conditions such as temperature (from ten days to several months). Pupation, like larval development, can vary from weeks to months, with the pharate adult occurring after approximately one day (Barros-Cordeiro *et al.,* 2014).

Adult size is closely linked to larval feeding (e.g. Addeo *et al.,* 2021). Adult male and female flies look similar, though males are often smaller in body size (Jones and Tomberlin, 2019). Unlike the well-developed larval mouthparts, which allow them to consume numerous substrates during development (Kim *et al.,* 2010), adults have sponging mouthparts for consuming liquids (Oliveira *et al.,* 2016; Rozkošný, 1982). Depending on the availability of food and water, BSF adults may live between 8-73 d (Nakamura *et al.,* 2016); however, adult BSF do not need to feed in order to reproduce (Sheppard *et al.,* 2002).

BSF mating can occur on the ground (Tingle *et al.,* 1975) or in flight (Copello, 1926). Males may be attracted to 'calling' females (Tingle *et al.,* 1975) or engage in lekking behaviours, with several hundred males congregating in an area (which may serve as an attractant to females; Tomberlin and Sheppard (2001)). Males at leks grapple with one another in the air, until one leaves the area; males also chase and grab females mid-air. Morphological, neuroanatomical, and behavioural data indicate males use a variety of senses, including visual, chemical, and/or acoustic cues, to locate females (Barrett *et al.,* 2022; Giunti *et al.,* 2018; Heussler *et al.,* 2018; Holmes, 2010; Klüber *et al.,* 2020; Liu *et al.,* 2020; Macavei *et al.,* 2020; Nakamura *et al.,* 2016; Oonincx *et al.,* 2016; Schneider, 2020; Tingle, 1975; Tomberlin and Sheppard, 2002; Zhang *et al.,* 2010).

In captive mating cages, males have been shown to engage in aggressive mating 'balls' of males with 4-12 individuals in larger enclosures. Larger-bodied males tend to be more aggressive and successful at mating (Jones and Tomberlin, 2021). Male-female courtship rituals may follow a stereotyped sequence detailed in Julita *et al.* (2020), which can include elements of female choice (Giunti *et al.,* 2018). Females may mate more than once (in captive conditions; Jones and Tomberlin (2021)). However, presumably-mated females at ovipositing sites with males present in the wild are not chased, indicating some external cue that demonstrates their unavailability for additional mating attempts (Tomberlin and Sheppard, 2001). Females lay eggs in dry crevices above food sources, with approximately 600-700 eggs/clutch (and one clutch, typically, during her life); unmated females also lay unfertilised eggs.

BSF have a highly adapted immune system that allows them to live in microbe-rich waste substrates such as animal manure. Transcriptomic studies have identified 57 active anti-microbial peptides (AMPs) in the haemolymph (Moretta *et al.,* 2020; Vogel *et al.,* 2018). These AMPs effectively limit the growth of certain bacteria, with probable effects on specific fungi and viruses currently unexplored (Alvarez *et al.,* 2019; Liu *et al.,* 2008; Lopes *et al.,* 2020; Moretta *et al.,* 2020; Park *et al.,* 2015; Vogel *et al.,* 2018). The expression and antimicrobial activity of these AMPs are dependent on diet (high induction with diets supplemented by plant oils and protein), temperature, feeding regimen, and the presence of other microbes (Liu *et al.,* 2008; Lopes *et al.,* 2020; Vogel *et al.,* in press). The anti-microbial properties of BSF larvae may also have beneficial effects on the immune systems of livestock consuming them (De Souza Vilela *et al.,* 2021).

Industry-rearing conditions

Industry-rearing conditions vary widely, as producers must navigate population- and location-level variation while advancing the technologies used in their facilities; few resources are currently available documenting this variation. Below is a brief overview of some of the industry practices and, where possible, determinations about what is likely to be most common in the industry at this time.

Larval rearing practices

Eggs can be hatched in a 'nursery' setting, which helps prevent the spread of pathogens to older larvae. Some nurseries provide 'special, high quality diet' to neonate larvae, though the contents of this mix is generally unspecified (Yang, 2019; but starter chick feed is used with 70% water content in Dortsman *et al.,* 2017). The majority of egg hatch failure in industry settings is attributed to 'high heat and low humidity' suggesting that managing these conditions in facilities can be challenging. Females may also be unmated, or interrupted in the process of mating, resulting in unfertilised eggs that will never hatch (inefficiencies in mating appear to be common; Tomberlin, 2018).

Larvae are currently fed vegetable, dairy, and bakery by-products, spent brewers' or distillers' grains, and animal feeds, with short-term plans to expand into food surplus and animal by-products. Long-term plans for BSF propose feeding them manure, municipal waste, and slaughterhouse, meat, or fish waste, though these may present safety concerns for organisms consuming the final BSF as a product (Bessa *et al.,* 2021; EFSA, 2015; FAO, 2021).

All waste provided to larvae must first be processed as a means to optimise the system and ensure substrates provide appropriate conditions for larval rearing. This may involve:

dewatering/rewatering (changing moisture levels to create optimal rearing conditions), testing for environmental pollutants detrimental to larval survival, grinding substrates to reduce particle size, blending multiple substrates to create a more heterogeneous and balanced diet, and even fermenting/inoculating substrates with microbes to improve nutrient content, 'shelf life' of the wastes, and decomposition rates (Dortsman *et al.*, 2017; Yang, 2017b).

Producers may use either small, stackable pans or large rearing troughs to rear their waste-processing larvae (Yang, 2017b). For the pan method, substrate and 5-day-old (DOL) larvae from the nurseries are added together to the 'larveros' for waste processing. A small subset of 5-DOL larvae will be retained in the nursery, to be used as eventual breed stock. Excess 5-DOL are discarded (method not described). Approximately 40,000 larvae are given 1 m^2 space, distributed across several pans, for a density of ~4 larvae/cm^2 (Dortsman *et al.*, 2017). Substrate depth is generally no greater than 5 cm, in order to avoid unprocessed waste at the bottom. Food may be given once as a lump sum, but more frequently is provided incrementally (a feeding every 3-5 d) in order to prevent rot and overfeeding. Automated systems (Yang, 2017b) have decreased the labour costs associated with incremental feeding.

Larvae reared in pans are typically harvested using sieves which allow sorting of the larvae by size. Days prior to collection, active ventilation of the substrate reduces the moisture content and makes the drier, crumbly substrate easier to separate from the larvae. Automated or manual sieves may be used, but automated systems are favoured due to reduced labour costs and higher shaking frequencies that minimise larval product loss (Dortsman *et al.*, 2017).

Larger rearing troughs are more traditional, and typically rely on a continually feeding system. Unlike in pans, larvae are of different ages and the trough is continually stocked with new eggs and substrate. Prepupa self-harvest by crawling up a ramp and into a collection bin (Dortsman *et al.*, 2017), where most will be slaughtered but some will be allowed to pupate as breeders. The entire trough must occasionally be emptied, cleaned, and refilled. Commercially upscaling this traditional method has proved incredibly difficult, and the pan method (while more labor intensive) appears to be more common in industry.

Little information on larval survival in rearing facilities is available. However, Dortsman *et al.* (2017) provides data from one facility in Indonesia:

- 70% of laid eggs hatch (30% unfertilised or dead);
- 70% of larvae will pupate;
- 80% of pupae will emerge as adults (if provided the opportunity).

These data suggest there is significant room for BSF welfare improvements in industry settings.

Adult rearing, mating, and oviposition

Pupae are held in dark cages, where they will eventually eclose. Resulting adults can disperse in the cage to the open area where sunlight, or artificial lights, are available for mating. Emerging adults may be held in darkness for several days before being given access to the well-lit mating cage, in order to prevent mating and egg laying and keep a source of 'fresh reproductives' available. Small quantities of ethyl acetate may be used to paralyse adult flies in order to gather weight measurements (Dortsman *et al.*, 2017).

Adults are reared in mesh cages (typically between 4.26 and 19.5 m^3) probably in quieter, out-of-the-way locations to avoid mating interruptions, either inside greenhouses for natural lighting or with LED lights (often specially designed for BSFs). Adults may sometimes be provided with artificial or real plants in their mating cage to encourage lekking behaviours. Densities of adults are not typically listed, but based on industry experience, adult fly numbers can be in the thousands per cage (Tomberlin, pers. comm.). They are provided with brown or white cardboard/wood or sometimes plastic ovitraps ('eggies') to lay eggs in and are typically provided only with water (often in a shallow fountain or via a misting system to prevent drowning if too much water is present) and no food. Adults mate around two days old, oviposit around four days (Tomberlin and Sheppard, 2002), and survive a total of only 8-14 days (Tomberlin *et al.*, 2002a), much shorter than in lab studies with different nutritional/abiotic conditions. Moist, rotting food, sometimes seeded with conspecific larvae, may be provided to stimulate oviposition, similar to natural conditions (Booth and Sheppard, 1984).

It is possible to rear adults in the same space as larvae (Fischer and Romano, 2020) but this appears to be uncommon in the industry (but see Yang (2017a), where small larvae are seeded into rotting food left in adult cages to encourage adults to lay eggs).

Slaughter

BSF are generally slaughtered in the later stages of immature development (though adults may be slaughtered prior to cleaning and restocking mating cages, but in much smaller numbers). Larvae may be removed from food for 24-48 hours prior to slaughter (known as a 'feed withdrawal period') in order to void larval gut contents. Producers may also include a washing step that uses water to rinse contaminants off the larval surface (Larouche, 2019). Current methods of slaughter for BSF larvae include: freezing (in air or liquid nitrogen), baking in a convection oven, roasting in sand or sunshine, microwaving, boiling/

blanching, asphyxiation, and grinding/shredding (EAWAG, n.d.; Larouche *et al.,* 2019; Mat *et al.,* 2021). BSF larvae may be sold prior to slaughter and consumed live by pets or livestock without anaesthetic.

Variation in slaughter methods used may result from expense, knowledge, equipment availability, batch size, or desired product qualities. For example, microwaving produces 'puffed' larvae that may be more desirable to consumers than the harder, smaller larvae produced through convection drying (EAWAG, n.d.). It should be noted that slaughter method will also impact nutritional makeup (Huang *et al.,* 2018), as well as lipid oxidation and microbial contamination levels, and the colour of the final product (Larouche *et al.,* 2019; Zhen *et al.,* 2020). Very little data are available on the specific methods used by producers, but freezing in air, blanching (for whole insects; Van Huis and Tomberlin, 2017), and grinding (for powdered insects) may be common.

2. Rearing conditions and black soldier fly welfare

BSF welfare during rearing can be impacted by: interspecific interactions (pathogens, parasites, and predators), abiotic conditions, nutrition, injury and crowding, handling-associated stress, selective breeding and genetic modification, and environmental contaminants.

Interspecific interactions

Pathogens and disease

Disease outbreaks are common in many farmed invertebrate populations and can result in significant mortality, as well as symptom-related suffering and loss of natural behaviours before death. Apparent symptoms of insect diseases vary based on the pathogen, but may be associated with suffering prior to potential mortality, including behavioural changes, lethargy, sepsis, swollen abdomens, feeding cessation, and more (Joosten *et al.,* 2020).

BSF may be less likely to experience large-scale disease outbreaks compared to other farmed insect species. Not a single large-scale disease outbreak had been reported by 20 international BSF producers as of 2015 (Eilenberg *et al.,* 2015), and the larvae may be more resistant to pathogens due to their specially adapted immune system (Joosten *et al.,* 2020; Zdybicka-Barabas *et al.,* 2017). However, anecdotal accounts by BSF producers suggest pathogens are a more serious concern than currently reported in the literature: 'there are unpreferable fungi that could hurt the colony… and some attacks will result in population wipeout… A virus infection usually kills over 90% of the larvae' (Yang, 2017a) and 'there is a good chance you will experience a disease outbreak. Some may even say it is inevitable… [you will] understand the frustration in losing some or all of your colony' (Miranda, 2021).

As BSF facilities continue to scale up their production, disease outbreaks and observations of novel pathogens in the industry are more likely. New insect viruses are continually being discovered (e.g. Patterson *et al.,* 2020; Yang *et al.,* 2016), and high stocking densities associated with low genetic variation (common in farmed insect populations) can lead to the emergence of highly pathogenic diseases (Maciel-Vergara and Ros, 2017; Steinhaus, 1958). Researchers suggest that the low number of fungal pathogens reported for BSF compared to other Diptera is due to a lack of study/sampling, and not a lack of existence, as almost all insects naturally host entomopathogenic fungi (Joosten *et al.,* 2020; Meyling and Eilenberg, 2007).

Several sources focused on rearing BSF larvae in the lab or in production facilities mention issues with fungal pathogens for both early- and late-stage larvae (Sheppard *et al.,* 2002; Yang, 2017a). BSF are known to be susceptible to *Beauveria bassiana* fungal infections (Lecocq *et al.,* 2021), and *Entomophthora* species have been recorded infecting Stratiomyidae (though the species of fungus and fly are not identified; Balazy (1993); Eilenberg (2000)). Barrett (pers. comm) received a batch of fungi-infected pupae from a producer in 2021 which caused 100% mortality (unidentified fungus, Supplementary Figure S1). A producer's video from 2016 describes unidentified fungal infections induced in pans of larvae, causing them to move slowly, stop eating, and eventually die. The video also details an untreatable species-specific disease that kills larvae slowly, preventing both metamorphosis and eclosion (Symton BSF, 2016). An informal report (Lecocq, 2019) documented twisted pupae that failed to eclose and adults with deformed wings, both due to unknown causes but suggestive of viral infection (e.g. deformed wing virus in *Apis mellifera,* L. (Hymenoptera: Apidae) Lanzi *et al.* (2006)).

As new larval food sources with novel microbiota are explored, pathogens may be introduced to production facilities. Joosten *et al.* (2020) reviewed the most likely fungi, bacteria, protozoa, and viruses of importance for future study in BSF. In addition to their review, *Penaeus merguiensis* densovirus (PmergDNV), which transmits across farmed invertebrate taxa (from crabs to crickets), may be of importance for BSF (Fauce and Owens, 2008). Researchers should focus on identifying pathogens that may threaten BSF before outbreaks occur, in order to implement preventative measures and awareness programs for producers. Given that fungal infection has been reported by numerous sources for farmed/lab-reared BSF, and that fungi like *Entomophthora* have a long history of co-evolution with Diptera (Jensen and Eilenberg, 2001; Jensen *et al.,* 2009), researchers should focus first on understanding BSF susceptibility to fungal species that are: (a) generalists common in other farmed invertebrate species; (b) likely to be found in substrates used for rearing BSF larvae (for example, due to use in biocontrol); or (c) known to infect other dipterans. Only two of the 57 AMPs identified for BSF have anti-fungal properties (Moretta *et al.,* 2020), suggesting BSF may be particularly susceptible to pathogenic fungi.

Researchers should also study the conditions that may cause latent and asymptomatic viruses to become pathogenic, generating sudden, severe, and persistently lethal outbreaks in farmed invertebrate systems (Eilenberg *et al.,* 2015; Weissmann *et al.,* 2012). Similarly, under stressful or overcrowded conditions, microsporidia can cause epidemics of slow-acting chronic infections resulting in physical deformities, slowed development, and early mortality (Becnel and Andreadis, 2014; Bjornson and Oi, 2014; Franzen *et al.,* 2005; Stentiford *et al.,* 2016). Identifying the precise conditions that facilitate these shifts in pathogenicity or virulence for microorganisms is another important future direction of research. While lethal diseases are of highest priority, non-lethal outbreaks may also impact BSF welfare.

Producers can take steps to manage potential disease risks, even while diseases remain largely uncharacterised (Eilenberg *et al.,* 2015, 2018; Joosten *et al.,* 2020; Table 4). Finally, rearing insects in conditions that mimic their natural environment as much as possible may be important for preventing disease outbreaks and facultative pathogens (Erens *et al.,* 2012), as well as rearing insects in abiotic conditions that are not conducive to pathogen development (e.g. lower humidity to deter fungal pathogens; Eilenberg *et al.* (2018)). More details on these and other disease prevention measures can be found reviewed in Eilenberg *et al.* (2015, 2018), and Joosten *et al.* (2020).

Parasites and parasitoids

In addition to diseases, other parasitic organisms may impact farmed BSF welfare, including mites (Acari), phorids (Diptera: Phoridae), nematodes (Nematoda), and wasps (Hymenoptera), which all impact other Diptera (Khoobdel *et al.,* 2019; Müller *et al.,* 2017; Perez-Leanos *et al.,* 2017). Unidentified 'red mites', which may be predatory on BSF larvae or simply commensal, have been documented on larvae reared in composting bins in Texas, USA (Müller *et al.,* 2017) and mites have been anecdotally reported on adult flies in the Northwestern United States. Flies covered in mites were observed to be more lethargic and spent large amounts of time attempting to clean mites from their bodies unsuccessfully (Jetson, 2020). Yang (2017a) noted that mites may come in with grain-based substrates and 'annoy' BSF of all life stages.

BSF pupae received from a producer by Barrett (pers. comm.) in 2021 came with an unidentified generalist mite species that covered both the pupae and the dermestid beetle larvae that arrived in the same container (Supplementary Figure S1). *Macrocheles subbadius* (Mesostigmata: Macrochelidae) is a generalist mite known to extract haemolymph from adults of other fly species, reducing their fitness/body condition and interfering with mating (Beresford and Sutcliffe, 2009; Perez-Leanos *et al.,* 2017; Polak, 1996, 1998; Polak and Markow, 1995). The presence of mites has been shown to extract an energetic cost from fly hosts, even when not yet infected (Luong *et al.,* 2017), and the effects of *M. subbadius* can be considered equivalent to starvation in some conditions (Polak, 1996). Mites threaten other mass-reared dipterans – namely, *Drosophila* (Diptera: Drosophilidae) fruit flies (University of Cambridge, 2021) – and have even been explored as biological control agents for house flies (e.g. *Macrocheles muscaedomestica* (Mesostigmata: Macrochelidae), Ho (1985); Safaa *et al.* (2014)). Grain mites are other common co-habitants of *Drosophila* and may impact BSF stocks feeding on grains as well. While not directly predatory on BSF, grain mites can breed rapidly to large numbers, potentially competing for food with larval BSF (Müller *et al.,* 2017). Phorid flies, similarly, are not directly predatory but compete for food with larval BSF and can breed to large numbers, making rearing facilities dirtier (Yang, 2017a) and potentially spreading disease between batches/pans.

Entomopathogenic nematodes have been explored as biocontrol agents for dipteran pest species, with some nematodes killing up to 80% of larvae, pupae, and adults (Torrini *et al.,* 2020). Many nematodes have broad host tropism, such as *Heterorhabditis* species (which infect house flies; Shapiro-Ilan *et al.* (2009)). *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) is capable of BSF infection (Tourtois *et al.,* 2017). While many nematodes require high humidity, and have low survival in certain kinds of manure (reducing the probability of house fly larval infections; Georgis *et al.* (1987)), BSF larval substrates often have 'excessive moisture' (Cheng *et al.,* 2017), potentially increasing the probability of nematode infection. Nematode infection can act quickly to produce high levels of mortality in larvae (95% within six hours, in some cases, but more often approaching 100% after two days; Georgis *et al.* (1987), Sharpiro-Ilan *et al.* (2009)) and BSF larvae have been shown to be susceptible to numerous nematode species, particularly in prior to the sixth larval stage (Tourtois *et al.,* 2017).

Parasitic wasps (Hymenoptera) used to control pest dipteran populations agriculturally may also target BSF (Tomberlin, n.d.) if brought into rearing facilities with substrates used to feed larval populations. While few specific species of wasps that impact BSF have been identified, Pteromalidae wasps are likely parasites of BSF larvae and/or pupae given their use of house (*Musca domestica* L. (Diptera: Muscidae)) and stable fly (*Stomoxys calcitrans* (L.) (Diptera: Muscidae)) hosts (Skovgård, 2002; Tomberlin, n.d.; University of California Riverside, n.d.). *Trichopria* sp. (Hymenoptera: Diapriidae) have been observed targeting pupating BSFs on farms at rates of 21-32% in summer months (Bradley *et al.,* 1984; Mitchell *et al.,* 1974), and have been found in industrial rearing facilities where they 'occur in large numbers and… damage the pupate emergence rate' (Yang 2017a). In addition, the generalist parasitoid wasp species *Dirhinus giffardii* (Hymenoptera: Chalcididae) was found in a rearing facility in Ghana, killing up to 71% of pupae in a batch, with an average of 52% mortality (Devic and Maquart, 2015).

Controlling mites (and nematodes) involves many similar measures to controlling other diseases: quarantine/cleaning for any new stocks/substrates brought into the facility, cleaning rearing bins, hands, and tools before use with a new population of larvae, and regularly cleaning detritus/debris in the rearing facilities to prevent mite and phorid populations from becoming established outside the rearing bins and re-entering rearing facilities (University of Cambridge, 2021). Mite/nematode infected bins should be immediately isolated and cleaned; in the case of mites, it is possible larvae may be cleaned and transferred to new substrates, but they should still be kept quarantined from other larvae until the infestation is under control. Controlling parasitic wasp infestations may be more challenging given their heightened mobility – however similar methods of isolation should be employed; if the wasp is a pupal-stage only parasite, it may be possible to control the infestation by simply isolating all pupae before thoroughly cleaning the rearing facilities. Sticky tape traps are also currently in use for controlling parasitic wasps (Yang, 2017a) and, likely, adult phorids (though these traps pose a welfare concern for the parasitic insects they manage).

Predators

In general, predators are likely to be of low significance for farmed BSF; however, ants (Hymenoptera; Chia *et al.* (2018)), spiders, (Araneae) and cockroaches (Blattodea) may also predate upon larvae (Holmes, 2010; Holmes *et al.,* 2017). Nonlethal products that disrupt insect movement (such as Insectaslip/Fluon) should be used to prevent pathing between possible nesting sites and rearing bins; most arthropod predators may be deterred from accessing larvae through mesh bin covers or by placing the legs of the rearing tables/stands in buckets of soapy water.

Abiotic conditions

Abiotic stressors can lower animal welfare both through direct effects or by combining synergistically with biotic/abiotic stressors to worsen an organism's condition (Polak, 1998).

Temperature

Ectotherms like insects are particularly reliant on environmental temperatures to maintain an optimal body temperature due to their reduced metabolic rates and often smaller body sizes (Huey and Stevenson, 1979; Wieser, 1973). Organisms typically have a range of survivable temperatures (with a much larger window of cool vs high temperatures), with a narrower band of optimal/preferred temperatures (Sinclair *et al.,* 2016). Living outside this preferred range can generate potentially adverse developmental, physiological, or behavioural impacts for insects (Harvey *et al.,* 2020). However, it should be noted that preferences for select abiotic or biotic factors can be population specific. Producers should determine how various abiotic factors influence the welfare of their specific population when mass produced.

The lower temperature threshold for BSF egg hatch is between 12 and 16 °C; eggs at 12 °C and lower were non-viable (Chia *et al.,* 2018; Holmes *et al.,* 2016). Temperatures of around 16 °C delay egg hatch to 15 days (Holmes *et al.,* 2016), while egg hatch normally takes 4-5 days at 24-25 °C (Booth and Sheppard, 1984; Chia *et al.,* 2018). In addition, lower temperatures reduced the percentage of hatched eggs from 75% at 19 °C to only 11-13% at 15-16 °C (Chia *et al.,* 2018; Holmes *et al.,* 2016). The lower threshold for larval development is affected by population (Dixon *et al.,* 2009), acclimation period, and diet (Spranghers *et al.,* 2017), but has been determined to be between 16 and 19 °C for BSF from Texas, USA (Holmes *et al.,* 2016) and between 10 and 15 °C for BSF in Kasarani, Kenya (Chia *et al.,* 2018).

Low temperatures also delayed development, and reduced survival. In the Texas population, a temperature of 19 °C increased time-to-eclosion to 72 days; only 32% of flies survived to adulthood. 100% of the larvae failed to survive a developmental temperature of 16 °C (Holmes *et al.,* 2016). In the Kenya population, larvae reared at 15 °C took 181-184 days to develop, with reduced survival at each life stage: 83-87% for larvae, 59-65% for pre-pupae, and 49-62% for pupae (Chia *et al.,* 2018). Variation between populations may be due to genetic variability (such as the differences in development time and growth observed across strains in Zhao *et al.* (2013), and the high genetic variability across populations documented in Ståhls *et al.* (2020)), or other differences in rearing conditions that were not controlled in these studies (e.g. humidity, etc.) that have individual or synergistic effects on fly survival.

The upper threshold for BSF egg hatch is between 40 and 42 °C, although viability drops precipitously from 75 to 10% between 35 and 37 °C. 100% of larvae are unable to survival to adulthood at temperatures between 37 and 40 °C. Development time was either longer, or not affected, when temperatures exceeded 30 °C (depending on diet; Chia *et al.*

(2018)). Development time was lowest, and larval survival highest, at 30 °C (Chia *et al.,* 2018; Tomberlin *et al.,* 2009). Differences in thermal maxima data across studies may be due to the selective effects of rearing colonies at particular temperatures or differing methodologies; for example, Tomberlin *et al.* (2009) found that 36 °C was the maximum temperature for larval development while Lardé (1989) determined larvae could withstand at least 40 °C; Chia *et al.* (2018) estimate the thermal maxima is between 37.2 and 44.0 °C, and Newby (1997) determined it to be 47 °C.

Adults differ from larvae in their optimal temperature range. Adults reared at 27 °C lived longer than those reared at 30 °C (Tomberlin *et al.,* 2009). Other studies found that longevity is increased for males and females around 20-25 °C, with no impact on the number of eggs laid at 20 °C, despite an increased pre-oviposition time (Chia *et al.,* 2018). These data contrast previous field data that suggest nearly 100% of oviposition occurs between 27.5-37.5 °C (Booth and Shepherd, 1984). Studies of critical thermal maxima suggest that 44-46 °C is lethal for adults (Laursen *et al.,* 2021). Age and size (but not sex) determined preferred temperature for adults: young adults (1 day after eclosion) preferred warmer temperatures (28.7 °C) than 7-day-old adults (19.2 °C), while smaller adults preferred cooler temperatures than larger adults (Addeo *et al.,* 2021). Notably, adults were not fed in Addeo *et al.* (2021); thus, negative correlations between age and preferred temperature may be related to reducing metabolic rate under starvation conditions, and this result should be re-examined with fed adults.

Taken together, these data suggest that rearing BSF below 19 °C or above 30 °C can result in significant mortality (or reduced longevity) and should be avoided. There is the possibility for significant variation among populations of BSF or even interactions between diet, population, and temperature, however the optimal temperature range is likely always close to: 25-30 °C. Providing a gradient of temperatures for adults and larvae within their preferred range may allow individual thermoregulation via microclimate choice.

For BSF larvae, crowding, combined with metabolic heat production and aerobic fermentation by microbes in the substrate, can cause BSF to experience high temperatures even in seemingly well-regulated environments (Lardé, 1989). Unpublished data reported in Yang and Tomberlin (2020) suggests that actively feeding larvae in a room temperature (25-28 °C) plastic rearing bin produce enough collective metabolic heat to reach 42 °C – a temperature that is lethal to 100% of eggs and larvae if experienced for a sustained period (Chia *et al.,* 2018). Managing heat in larval rearing areas is thus a critical welfare intervention for farmed BSF.

Limiting the number of larvae per unit of feed, putting mesh barriers within bins to separate larvae, or creating feeding bins with high surface area to volume ratios that reduce larval crowding within the substrate, all may help prevent overheating. Reduced larval rearing densities result in higher survival, larger body mass, and slower development times (a 10% increase in survival when comparing 1-2 larva/cm^3 versus 10 larvae/cm^3; Dzepe *et al.* (2020), and see Barragan-Fonseca *et al.* (2018), Paz *et al.* (2015)). Other thermoregulatory measures such as convective cooling units or spraying cool water onto the feed regularly might also be successful in reducing temperatures. Finally, temperatures should be monitored broadly within the rearing facilities to check for unequal heat distribution throughout the facility (e.g. temperature stratification), which may impact bins of larvae or cages of adults stored in those areas.

Humidity/moisture

BSF evolved in the tropics where relative humidity (RH) is frequently quite high (an annual mean of 90% in Brazil; Silva and Souza (2016)). However, many producers of BSF are located in temperate zones with reduced RH, which may subject flies to desiccation stress. Different BSF life stages may be differently susceptible to desiccation stress based on their microenvironment (eggs, post-feeding larvae, pupae, and adults have higher contact with air than feeding larvae, so RH may be more critical) and respiratory/cuticular physiology.

RH positively correlated with oviposition by adult BSF females: 80% of eggs were laid when RH was above 60% (RH ranged from 30-85%; Tomberlin and Sheppard (2002)). Reduced oviposition at lower RH may be to prevent egg desiccation (Tomberlin *et al.,* 2002a). Eggs kept at 25% RH saw >90% mortality and delayed eclosion (Holmes *et al.,* 2012); however, such a low RH is typical only in arid regions (e.g. Arizona or Nevada in the United States). However, egg hatch was correlated strongly with RH – even at 50% RH, >60% of eggs did not hatch (compared to 14-35% at 70% RH; Holmes *et al.* (2012)). One producer's rearing guide recommends an RH around 50% in mating cages – the effects of this relatively low humidity on mating/oviposition rates and egg hatch are not discussed (Yang, 2017a). Humidity may also affect the flight abilities of male flies engaged in mating activities by impacting the flexibility of their halteres (Parween and Pratap, 2015); more studies are need that assess the impact of RH on the ability to perform natural mating behaviours in cages of different sizes.

Post-feeding-larval and pupal mortality were significantly increased at 25 and 40% RH compared to 70%. Only ~2-3% of pupae and post-feeding larvae died at 70% RH (vs 62-65% diet at 25% RH). Adult longevity decreased from nearly 8 days at 70% RH to only ~6.5 days at 40% and ~5 days at

25% RH (Holmes *et al.,* 2012). Guides for rearing BSF note their resilience to a wide range of RHs (30-90%; Park (2016), Sheppard *et al.* (2002)); however, if increased mortality is taken as an indicator of poor-welfare conditions, the data in Holmes *et al.* (2012) suggest that eggs, pupae, post-feeding larvae, and adults experience reduced welfare when RH was lower than 70%. Thus 70% should be considered the minimum relative humidity for rearing BSF. Future studies looking at health, behaviour, and survival between 60-90% RH would also be valuable, while taking into account the possibility for population-level variation in RH tolerance.

Feeding larvae bury themselves in their food substrate, and will instead be impacted by the moisture content of the substrate. Substrate moisture impacts not only desiccation stress but also feeding behaviour and larval movement by altering oxygen diffusion (see subsection 'oxygen') and substrate texture (Makkar *et al.,* 2014; Palma *et al.,* 2018). For example, chicken feed kept at 45% moisture (w/w) developed clumps at the surface, forcing larvae to the bottom 50% of the substrate; chicken feed kept at 75% had a thin water film at the surface and larvae were found only in the upper half (55-65%, larvae were found at all depths; Bekker *et al.* (2021)).

Ideal moisture levels are highly dependent on the substrate. BSF larvae have been reported to develop at moisture contents of 10.0-97.5% (Fatchurochim *et al.,* 1989; Lalander *et al.,* 2020), but moisture contents between 60-80% generally appear to be optimal for development, body weight, and survival (Bekker *et al.,* 2021; Cammack and Tomberlin, 2017; Cheng *et al.,* 2017; Dzepe *et al.,* 2020; Palma *et al.,* 2018). For example, at 85% moisture in chicken feed, 100% of larvae died – compared to only 19% at 75% moisture and 5-11% at 45-65% moisture (Bekker *et al.,* 2021). However, ~40% moisture in almond hull substrates or other cellulose-based diets led to 100% larval mortality while moisture from to 50-70% increased growth and final larval weights (Cammack and Tomberlin, 2017; Palma *et al.,* 2018). A third study in food waste found >95% survival at moisture levels between 75 and 85% (Cheng *et al.,* 2017). These data suggest that ideal moisture levels are highly dependent on substrate characteristics and should be experimentally determined for any new substrates.

Studies have reported larval drowning at high moisture contents in low porosity substrates, even with active ventilation (Lalander *et al.,* 2020). Moisture may be managed by adding wheat bran or sawdust around the outer edge of substrates to wick away excess moisture from the base, while new moisture may be added to the top (Yang, 2019), or via the addition of coco peat to the substrate surface to reduce moisture loss (Dortsman *et al.,* 2017).

High RH/moisture can increase pathogen/parasite levels – for example, increased fungus development occurred in high humidity chambers of neonates (Sheppard *et al.,* 2002; see subsection 'parasites'). In addition, low moisture levels appear to favour microbial decomposition of substrates over larval growth; this competitive interaction may reduce larval welfare if larval growth is considered an important indicator (though survival was generally unaffected; Bekker *et al.* (2021)). Careful attention should be paid to balance the numerous abiotic and biotic stressors.

Oxygen

Larvae typically feed in the top 2-4 cm of substrate, where oxygen is likely plentiful. However, some producers may provide deeper or very moist substrates, in which case aeration will be necessary to prevent zones of low oxygen with negative welfare implications. Manufacturers may manually churn substrates (e.g. using a shovel or rotary drum to turn the material and introduce air) or use forced aeration (increased substrate ventilation using fans, Lalander *et al.* (2020), or tubing distributed throughout the substrate that releases gas at a specific rate, Palma *et al.* (2018)). Active ventilation could be used to increase oxygen diffusion, although according to Lalander *et al.* (2020), it reduces moisture content of the substrate more quickly than passive ventilation. Drying associated with active ventilation can also cause larval death, as the substrate shrinks and larvae covered in residue become stuck to the sides of containers and dry out. Aeration of substrates can be challenging when deep substrates have high moisture contents or dense textures, slowing the diffusion of oxygen from the surface and creating anaerobic zones at the bottom of the substrate that larvae must avoid (Lardé, 1989). Generally, low oxygen conditions reduce successful larval development. Increasing substrate aeration from 0.04 to 0.36 ml/min/g tripled larval harvest weight; increasing aeration beyond 0.36 ml/min/g did not affect larval growth (Palma *et al.,* 2018).

Smaller larvae are particularly susceptible to high-moisture, low-oxygen environments as they are not as physically strong (Yang, 2017a), making it more challenging for them to wiggle through dense, wet substrates and create pores where they can breathe. Adding low density, high rigidity substrates (wheat bran, rice bran, wood shavings) as a means of managing moisture, creating a loose texture, and supporting passive oxygen diffusion will promote neonate survival. Reducing the depth of the substrate also allows larvae to resurface if deeper zones become anaerobic, reducing the likelihood of suffocation.

pH

Substrate pH may impact both the larvae and their gut microbiome, resulting in changes to survival and growth rates. BSF larvae tolerate a wide range of pH, growing at initial substrate pHs from 2 to 11. However, pH values of

2-4 were typically not optimal for growth and could reduce survival to the prepupal stage (Ma *et al.,* 2018; Meneguz *et al.,* 2018; Pang *et al.,* 2020). Substrates can become acidic (pH of 4-5) when too much substrate is provided, generating anaerobic conditions which produce acidic by-products of anaerobic breakdown (Paz *et al.,* 2015). BSF conversion of substrates ranging from in pH from 4 to 11 resulted in values consistently between 8.5-9.5 after 10 d, demonstrating that larvae are capable of modifying the environment's acidity via the by-products of their consumption (Meneguz *et al.,* 2018; Pang *et al.,* 2020).

Larvae typically grew to the largest size in neutral-to-basic initial conditions (Ma *et al.,* 2018; Pang *et al.,* 2020), although in some studies there was no difference in growth amongst conditions (Meneguz *et al.* (2018); although size is not necessarily a reliable indicator of welfare). Vegetable/restaurant/household waste and wet distillers' grain often have an acidic-to-neutral pH, but manure pH can depend strongly on the age, type, and number of animals producing the manure (from acidic to slight alkaline; Nahm (2003), Stewart *et al.* (2017), Sundberg *et al.* (2013)). In general, pH appears to be most affected by microbes inhabiting substrates.

Pupal substrate

Post-feeding larvae must seek an appropriate pupation substrate. Because larvae are typically harvested at this stage for slaughter, little research has addressed pupation substrate suitability; however, larvae reared for mating purposes must first undergo metamorphosis and therefore pupation substrates are still relevant to welfare in the industry setting.

Post-feeding larvae took the longest to begin pupation, and had the lowest survival to adulthood, when provided with no substrate (Holmes *et al.,* 2013). Reduced survival is likely related to increased energy expenditure wandering and searching for suitable substrates. Out of the four substrates provided, sand, topsoil, soil, and wood shavings, those with higher compaction (sand, topsoil) had increased time-to pupation and lower burial-depths for the pupae; this may be due to the physical challenge of digging through low porosity substrates, or these substrates may not be favourable due to decreased oxygen availability. Wood shavings also had the highest proportion of living adults following pupation, suggesting it may be the ideal pupation substrate given its high porosity (ease of movement, lots of oxygen) and moisture retention (reduced risk of desiccation) abilities (Holmes, 2010; Holmes *et al.,* 2013).

Both wandering and burial are 'natural behaviours' for this life stage. In systems where larvae are allowed to self-harvest, they are typically not given access to a pupation substrate and are denied the opportunity to engage in this natural behaviour prior to slaughter. Future research should address if post-feeding larvae, allowed to wander for certain lengths of time outside both a larval and pupal substrate, become stressed when denied the opportunity to find an appropriate pupation location. These data would inform slaughter schedules, post-feeding larval rearing container set-up, and also the provision of pupation substrates in shipments of live, post-feeding larvae to consumers.

Light

Light conditions can affect both developmental and reproductive behaviours. BSF larvae are photophobic (Canary *et al.,* 2009; Fisher and Romano, 2020; Newton, n.d.), and will move to attempt to escape light (Mat *et al.,* 2021). Larvae experienced a small delay in development when exposed to a growth chamber with 10.6% higher light intensity on a 12-12 hr photoperiod (Holmes *et al.*, 2012; similar to *Drosophila* larvae, Bruins *et al.* (1991)). Photoperiod may also have impacts on development: larvae reared in a no-light condition had increased post-feeding larval mortality and longer developmental times compared to 8:16 and 12:12 photoperiod conditions (but this may have been related to predation: Holmes (2010), Holmes *et al.* (2017)). Generally, as long as an appropriate feeding substrate is provided with sufficient depth, BSF larvae are capable of escaping most light. Producers may keep pupae in the dark, in order to stagger adult emergence, which does not appear to adversely affect pupae (but does adversely affect post-feeding larval development and survival; Holmes *et al.* (2017)).

Photoperiod and light type impact adult longevity and behaviour (reviewed in Awal *et al.*, 2022). Adults lived longer in no-light conditions compared to 12:12 photoperiods, though probably as a result of decreased activity levels (Holmes, 2010); it is possible that if adults were provided with adequate nutrition (see subsection 'adult nutrition' and Macavei *et al.* (2020)), longevity differences would disappear. Sunlight stimulates adult activity, including mating behaviour, with a minimum light intensity of 63 $\mu mol/m^2/s$ required and most mating occurring over 200 $\mu mol/m^2/s$ (Tomberlin and Sheppard, 2002). Lower sunlight intensities during winter or inclement weather can reduce mating significantly (Park *et al.,* 2010; Tingle *et al.,* 1975), although a few adults mated under no-light conditions in Holmes (2010). Increased light intensities led to increased mating (from 23 to 70%), as well as earlier onset of mating, in lab-reared BSF under LEDs with peaks at 440 and 540 nm (wavelengths to which some BSF photoreceptors are maximally sensitive; Schneider 2020). Conversely, Oonincx *et al.* (2016) found no differences in the number of larvae produced under different light intensities.

Light type is also considered to be important, but studies are similarly inconsistent – for example, while some studies reported no mating with artificial light sources (Tomberlin and Sheppard, 2002), but standard fluorescent lab lights with a 24-hr photoperiod supported mating behaviours (Fisher and Romano, 2020). Tests of multiple artificial light sources report population-dependent effects on mating success (Liu *et al.,* 2020). Overall, mixed LEDs (compared to white LEDs/neon lights; Macavei *et al.* (2020)), BSF-LEDs (compared to halogen, white LEDs, and metal halide; Liu *et al.* (2020)), or high-intensity sunlight (Compared to quartz-iodine lamps, rare earth metal lamps, and white LED + fluorescent combinations; Nakamura *et al.* (2016), Zhang *et al.* (2010)) are the best for BSF mating across multiple studies.

A lack of control of optical variables such as light flickering frequencies, intensity, colour temperature, photoperiod, spectra of irradiance, polarisation, or conflict with circadian rhythms (e.g. time of day) may account for the significant variation in mating behaviours under different light types, alongside differences in populations. Colour temperature has been found to impact peak oviposition periods: 'warmer' LED lights having earlier oviposition peaks than 'cooler' lights (Klüber *et al.,* 2020). In addition, conflating a 'laid egg' with a 'viable egg' also can make results hard to interpret across studies – studies that only look at egg clutch size variables, and do not directly test the viability of those eggs, may not be accurate reflections of the effects of light source on mating behaviour (Heussler *et al.,* 2018; Klüber *et al.,* 2020). Using the right light conditions is critical for encouraging natural mating behaviours for BSF, and thus is a welfare concern worthy of further investigation.

Multiple studies reference the energy efficiency of different lights for mating cages, suggesting the industry may currently be hampered by costs associated with providing optimal light sources for mating BSF indoors (either the costs of the lights, or the costs of heating and maintaining greenhouses to provide natural lighting; Heussler *et al.* (2018), Liu *et al.* (2020)).

Adults – spatial and oviposition needs

Numerous studies have reported challenges achieving successful mating for BSF adults due to the spatial requirements of their mating behaviours (Table 1), which includes courtship displays, chasing, territory establishment, aggressive territory defence, lekking, and copulation itself, much of which happens aerially (Giunti *et al.,* 2018).

Mating occurs, but is substantially reduced, in cages of approximately 1 m^3 or less compared to larger enclosures. Julita *et al.* (2020) notes that lekking behaviours and mating displays occurred more frequently in the larger 3 m^3 cages provided, as compared to the 1 m^3 cages. Behaviours observed in natural populations of BSF, such as male-male spiralling and grappling interactions (Tomberlin and Sheppard, 2001), were not observed in 1 m^3 cages containing only 10 males and 1 female (Giunti *et al.,* 2018). Although mating can be achieved in smaller cages, larger cages appear to allow more natural courtship behaviours to occur.

Few of the studies that mention spatial constraints also list the density at which they reared adults within the space provided, making their results harder to interpret. Nakamura *et al.* (2016) explicitly suggests that high adult density is critical to mating success under extremely low-space conditions (0.27 m^3). However high male density

Table 1. Effects of different mating cage sizes and light conditions on black soldier fly mating.

Study	Cage size (m)	Light condition	Result
Tingle *et al.* (1975)	3×6.1×1.8	outdoors	mating occurred
	0.76×1.14×1.37	outdoors	mating occurred
	0.76×1.14×1.37	greenhouse	no mating
	0.53×0.91×0.53	greenhouse	no mating
	0.38×0.46×0.38	greenhouse	no mating
Sheppard *et al.* (2002)	2×2×4	greenhouse	mating occurred
Tomberlin and Sheppard (2002)	1.5×1.5×3	greenhouse	mating occurred
Julita *et al.* (2021)	0.6×0.6×0.6	greenhouse	mating occurred
Julita *et al.* (2020)	1×1×1	greenhouse	mating occurred in both conditions; mating behaviour notably depressed in smaller cage
	3×3×3	greenhouse	
Yang (2017a)	1.2×1.2×1.2	greenhouse	mating occurred in both (industry conditions)
	1.2×0.64×0.51	w/LED (both)	
Heussler *et al.* (2018)	0.39×0.28×0.28	artificial lights	oviposition, yes, but mating not determined
Nakamura *et al.* (2016)	0.27×0.27×0.27	artificial lights	mating, only 11.2% fertilised
	0.27×0.27×0.27	sunlight	mating, only 39.5% fertilised

can cause increased aggression, and reduced longevity, in other Diptera (e.g. *Ceratitis capitata*, (Diptera: Tephritidae) Gaskin *et al.* (2002); *D. melanogaster,* Bretman *et al.* (2013)), and BSF and closely related *Hermetia comstocki* (Diptera: Stratiomyidae) males do engage in aggressive male-male encounters (Alcock, 1990, 1993; Tomberlin and Sheppard, 2001). While adult males naturally aggregate in high-density hot spots (leks), the ability to escape high density zones (and thus conflict) may be lacking in cages compared to field scenarios. Overall, it is likely that smaller mating enclosures may not allow for natural mating behaviours and thus represent a welfare, as well as production efficiency, concern.

Adult BSF may be very sensitive to interruptions during mating sequences (Yang, 2017a), suggesting mating cages should be placed in low traffic locations. Plants (real or artificial) in mating cages may encourage the lekking behaviours exhibited by male BSF in the wild but may not always be included in cages due to increased caretaking requirements (Tomberlin, 2017).

Studies of ovitraps have found that wood and cardboard are preferred (more female visits) to dry leaves and plastic (Julita *et al.,* 2021). Low-cost cardboard eggies are typical in the industry and do not represent a welfare concern. Ovitraps may be a source of pathogen proliferation, so disposable single-use or plastic, washable eggies should be used. Females may also prefer to oviposit on blue vs white surfaces (Romano *et al.,* 2020). Females may prefer to oviposit near substrates already inoculated with conspecific larvae (Furman *et al.,* 1959, unpublished data cited in Tomberlin *et al.,* 2002a), however more recent work has not supported this conclusion (Tomberlin *et al.,* 2002a). Still, females may be attracted to chemicals let off by microbes frequently associated with conspecific larvae (Zheng *et al.,* 2013). Additional observations of wild populations, as well as laboratory experiments, could resolve if attraction to conspecific larvae is an important ‘natural behaviour’ for the welfare of ovipositing BSF females.

Nutrition

Adult nutrition

Adults are usually provided only with water, often delivered through misting systems or fountains. Water access increases longevity in mated flies from about 8 d to up to 14 d (Macavei *et al.,* 2020; Tomberlin *et al.,* 2002a; Yang, 2017a).

Adult BSF are generally kept without food due to a widely held belief that they may live entirely off of energy reserves built during the larval stage (Caruso *et al.,* 2013; Sheppard *et al.,* 2002; Yang, 2017a). However, field observations suggest soldier flies do feed in the wild on honeydew, nectar, and possibly pollen and honey (Beuk, 1990; Copello, 1926; James, 1981; Oldroyd, 1973). Morphological examination suggests that their mouthparts meet all requirements for nectar feeding (Oliveira *et al.,* 2016; Rozkošný, 1982) and they possess a functional digestive system (Bruno *et al.,* 2019). Recent studies show adult BSF will ingest multiple liquid diets if provided; flies preferred honey over white or brown sugar-water or water alone (Romano *et al.,* 2020).

Beyond preference, BSF kept individually and not allowed to mate (mating can decrease longevity in Diptera; Chapman and Partridge (1996)), saw dramatic increases in longevity when provided access to sugar water – from 21-22 d (water-only) to 48-73 d (Nakamura *et al.,* 2016). Mated BSF also saw increased longevity when fed sugar water (Bruno *et al.,* 2019; Fisher and Romano, 2020; Macavei *et al.,* 2020; Oonincx *et al.,* 2016) or agar/milk, mixed with sugar (Bertinetti *et al.,* 2019). Currently, no studies have assessed protein vs carbohydrate-based (or mixed) food sources, nor the impact of including more natural sources such as pollen/nectar on adult BSF behaviour, reproduction, and longevity.

In addition, it should be considered that mating/mated males and females may benefit from different types of food sources based on the different energetic demands of their reproduction (Harrison *et al.,* 2012; Magwere *et al.,* 2004; Wong *et al.,* 2009). This may explain why sugar-water increased male vs female longevity, while protein-rich agar and milk increased female longevity in comparison to males (Bertinetti *et al.,* 2019; Nakamura *et al.,* 2016). The increased energy expenditure of female reproduction may also explain the reduction in female vs male longevity in most water-only conditions (e.g. Heussler *et al.,* 2018; Tomberlin *et al.,* 2009).

These data suggest that the current practices do not provide adult BSF with freedom from hunger, resulting in premature mortality due to starvation and representing significant potential for suffering. Feeding of adult BSF should not be considered supplemental, but obligatory, for animal welfare. Unfortunately, studies of adult nutrition have recommended providing only water (Macavei *et al.,* 2020) or nothing at all (Bertinetti *et al.,* 2019) in order to force faster oviposition and obtain the largest number of eggs over time. These practices do not provide freedom from hunger or thirst, irrespective of their effects on oviposition rates. It is also possible that more holistic diets (e.g. those containing proteins and sugars) may be best for egg viability/clutch size, and future studies should test for these and other effects of adult diets.

Food should be provided in mating cages using feeders, such as those developed for honey bees, that reduce feeder-associated mortality (via drowning or getting stuck). BSF preferred white feeders over yellow and green; brown, pink, and blue should be avoided (Romano *et al.,* 2020). Feeders should be changed/cleaned regularly to avoid pathogen spread. In this case, cage duration with flies in a facility would be based on egg production rather than adult longevity. Once egg production declined, the cage could be cleaned and restocked. Adult flies should be anaesthetised (see subsection 'use of anaesthetics prior to slaughter') before being humanely slaughtered (see section 'welfare during slaughter').

Larval nutrition

Larvae are capable of consuming most substrates, and naturally consume foods as variable as vegetable waste, carrion, coffee grounds, or human and other vertebrate faeces (Tomberlin *et al.,* 2005; reviewed in Diener *et al.,* 2009; Nguyen 2010). However, not all substrates produce similar effects on growth and survival. Additionally, rearing insects at industrial scales often poses additional challenges not present when using specific substrates (or abiotic conditions) in small-scale wild settings (reviewed for BSF in Miranda *et al.,* 2020; Scala *et al.,* 2020; see also Sørensen *et al.,* 2012; Yang and Tomberlin, 2020). Finally, populations, or 'strains', of larvae can vary significantly in their responses to substrates, complicating analyses conducted on different populations of flies, particularly when other abiotic variables are not kept constant (Zhao *et al.,* 2013).

The industry currently uses vegetable, dairy, and bakery by-products, spent brewers' grains, and animal feeds, with short-term plans to expand into food surplus and animal by-products. Long-term plans propose feeding BSF manure, and municipal, slaughterhouse, meat, or fish waste; however, to use these substrates safely, quality assurance measures need to be developed (EFSA, 2015; FAO, 2021). In most studies that list survival, different substrates present between 80 and 95% survival (but see information on human municipal waste, meat meal, and manure, below), suggesting that larvae are resilient to many different nutritional programs.

An extensive literature documents the impacts of diet on development time, body composition, and weight of BSF larvae; however, these data provide little information about welfare. Significant variation in abiotic conditions across studies, along with differences in pre-processing techniques that substantially alter the suitability of a substrate, makes generalising about the best or worst substrates for larval welfare very challenging. Even the 'same' substrate can vary in its nutritional composition based on geographic region, season, strain, and more (e.g. dessert vs juice banana peels yield a 4.5 fold difference in larval weights; Isibika *et al.* (2019)). However, a few general patterns emerge that may promote larval welfare.

Larvae perform best and have increased survival on heterogenous, high-nutrient content diets with a mix of carbohydrates and proteins (Cammack and Tomberlin, 2017; Gobbi *et al.,* 2013). Homogenous substrates with high hemicellulose and lignin contents are naturally high in non-digestible matter, and include: rice straw (Li *et al.,* 2015), manure (Miranda *et al.,* 2020; Nyakeri *et al.,* 2017; Rehman *et al.,* 2017a), spent brewer's grain (Lynch *et al.,* 2016), almond hulls (Palma *et al.,* 2018), paper waste (Tomberlin, pers. comm.), and some vegetable/fruit wastes (such as banana peel; Isibika *et al.* (2019)). Dairy manure reduced survival to 45% in Miranda *et al.* (2020), and ~71-77% survival in Myers *et al.* (2008). 100% dried distiller's grain with solubles (barley, corn, wheat and sugar syrups) led to a 34% reduction in survival (though this study had low survival rates in all conditions; Tschirner and Simon (2015)). Nutrient content is sometimes purposefully lowered when non-digestible matter is added to the substrate to increase rigidity/oxygen levels or manage moisture; these variables must be managed holistically.

Some substrates appear to be inhospitable to larvae, though data on their nutrient content is currently lacking. A diet of human faeces can dramatically reduce larval survival (under some feeding regimens, down to 8% survival; Banks *et al.* (2013)). Additionally, a diet of 100% 'meat meal' caused 60% larval and 80% pupal mortality (Gobbi *et al.,* 2013). Unless further research can determine processing regimens that promote survival and welfare in these substrates, they should be avoided for larval rearing.

Processing has the potential to improve larval welfare by altering substrate texture, microbe levels, pH, moisture, and nutrient composition. Grinding substrates may provide better textures for larval growth by reducing sharp edges (to avoid injury), increasing porosity, and generating loose texture (to improve movement, moisture retention, and aeration; Palma *et al.* (2018)); particularly, smaller larvae should be provided with smaller particles of food (Dortsman *et al.,* 2017; Tomberlin, 2017). Adding *Bacillus subtilis,* a bacteria isolated from BSF larvae, or other microbes that breakdown hemicellulose and lignin can improve the nutrient content of the substrate (Rehman *et al.,* 2019; Zheng *et al.,* 2012). Microbial inoculation can alter the favourability of other aspects of the substrate as well; for example, treating banana peel with microbes significantly increased the pH to a more favourable level for BSF growth (Isibika *et al.,* 2019). However, the effects of many different microbes on survival or welfare (as compared to growth) are poorly understood, and further research is needed to determine the suitability of different microbial inoculations when paired with unique substrates (Yu *et al.,* 2011; Zheng *et al.,* 2013). In addition, diet itself significantly impacts gut microbiota (Tanga *et al.,* 2021), with probable but currently underexplored impacts on BSFL health.

Substrates may be treated to improve heterogeneity and nutrient composition by mixing them with other substrates (glucose, plant-based oils, soybean curd residue, and restaurant wastes have all been used; Li *et al.* (2015), Rehman *et al.* (2017b), Vogel *et al.* (2018), Zheng *et al.* (2012)). While 100% chicken manure led to only 35% survival, adding just 20% dried food waste rescued survival to nearly 100% (Lee *et al.,* 2021). Similarly, mixing horse and sheep manure with vegetable waste improved survival by 11% (although the treatments were not statistically different) and increased final larval mass (Julita *et al.,* 2018). Larvae can tolerate a fairly large range of nutrient contents (potentially down to a 23% nutrient composition) but there are decreases in survival and large decreases in body size as a result (Barragan-Fonseca *et al.,* 2018). Maintaining at least 50% nutrient content in any substrate is recommended until more data can be collected.

Finally, producers should manage the amount of nutrition provided per larvae. Providing too much food can lead to anaerobic conditions and acidic pH (Paz *et al.,* 2015) as well as increasing the potential for pathogen proliferation (no more than 30-50% undigested before adding additional food; Tomberlin (2017)). While feeding larvae more food may produce larger body weights, it can also increase larval metabolic rates and the likelihood of overheating (Paz *et al.,* 2015).

Environmental pollutants

Some substrates may be more likely to be contaminated with pollutants that would harm BSF welfare, including entomopathogenic biocontrol agents (see subsections ‘pathogens’ and ‘parasites’), chemical pesticides or herbicides, toxins, and heavy metals.

Chemical pesticides and herbicides may be present on any unwashed plant-based agricultural waste, and may have lethal (e.g. neurotoxic pesticides, Costa *et al.* (2008)) or sublethal effects (De França *et al.,* 2017). Organophosphate pesticides, such as chlorpyrifos, chlorpyrifos-methyl, and pirimiphos-methyl did not impact BSF larval growth or survival (Purschke *et al.,* 2017). However, BSF larvae were found to be 12-31 fold more sensitive (LC_{50}) than house flies to some pyrethroids. 100% of BSF larvae die at half the typical concentration of cyromazine used to control pest dipterans. Pyriproxifen also reduced survival to adulthood (by a maximum of 32% at the highest concentrations) and may prevent larval pupation or sterilise resulting adults (Tomberlin *et al.,* 2002b). Manure, or other substrates commonly treated with these and other untested chemicals, should not be fed to BSF larvae until empirical tests can ensure they do not have adverse sublethal (or lethal) effects.

Crops that are genetically modified organisms (GMOs) may contain *Bt* toxins lethal to BSF (destroying the mid-gut epithelial cells, allowing bacterial colonisation of the hemocoel; Joosten *et al.* (2020)). *Bt* toxins bind in a taxon-specific manner, so BSF are most likely to be impacted by GMOs designed to control other dipteran pests of grain, fruit, and vegetable crops (Ogaugwu and Durvasula, 2017). Heat of 60-90 °C can be used to inactivate *Bt* toxins (Ujváry 2010), suggesting energy-expensive pre-processing of substrates followed by microbial innoculations may be necessary to ensure larval safety when feeding GMO substrates. Mycotoxins (common in some stored agricultural products) such as aflatoxins B1/B2/G2, deoxynivalenol, ochratoxin A, and zearalenone did not affect growth/survival compared to controls (Bosch *et al.,* 2017; Purschke *et al.,* 2017).

Heavy metals such as copper and cadmium have been shown to shift the larval gut microbiome, though they do not always affect larval weight gain or survival (Van der Fels-Klerx *et al.,* 2016; Wu *et al.,* 2020, but see Purschke *et al.,* 2017). Larvae are capable of accumulating numerous heavy metals, as well as arsenic, in their tissues (Diener *et al.,* 2015, van der Fels-Klerx *et al.,* 2016). At high enough concentrations this can reduce survival by up to 50% (Biancarosa *et al.,* 2017, but see Purschke *et al.,* 2017). These heavy metals may be found in municipal, household, and vegetable waste (Diener *et al.,* 2015). In addition, larval bioaccumulation of hazardous substances may have effects on animal livestock (as levels of accumulation in BSF larval tissue may exceed those considered safe for animal feed, Purkayastha and Sarkar (2021)).

Finally, some waste streams may contain ‘garbage’ that is detrimental to BSF survival: injurious sharp objects (such as glass), non-biodegradable matter (plastic bags, styrofoam cups), and acids/solvents/chemicals (batteries, detergents, etc., Yang (2017b)). These can be harmful to both human workers and BSF larvae. Some types are removed during waste pre-processing or, if they cannot be removed, the waste is not used (Dortsman *et al.,* 2017). However other kinds of trash, like plastics and cigarette butts, may make it into the larval food (Yang, 2017b) and can even be toxic to larvae (e.g. nicotine can be lethal to fruit flies, Wolf and Heberlein (2003)).

Genetic modification and selective breeding

Genetic modification (generally, using CRISPR/Cas9 technology) of BSF to optimise their use in industry may dramatically impact their welfare. Current genetic modifications under development include:

1. Increasing development time, and thus larval weight and feeding capacity, by knocking out the *Ptth* gene. *Ptth* encodes the prothoracicotropic hormone that initiates the signalling cascade resulting in moulting and metamorphosis (Fellner *et al.,* 2005). *Ptth* KO lines

saw 20-fold increases in the length of the last larval stage and a 2-3 fold increase in body weight (Zhan *et al.,* 2019).
2. Increasing the concentration of particular nutrients in the body of larvae, in order to provide optimal nutritional profiles for human consumption (details not listed, but project funding received by Echelon Biosciences, Gunther (2018)).
3. Creating flightless adults that can be housed in smaller cages, by silencing the *Vg* gene (Zhan *et al.,* 2019) which encodes for the size and shape of wings in *Drosophila* (Williams *et al.,* 1991).

Numbers 1 and 3 appear the most concerning for BSF welfare. Flightless adults clearly violate the freedom to express normal behaviour. The extension of larval feeding and dramatic increases in body size may actually decrease health and welfare, as well as potentially constrict normal behaviour (though no data have yet been provided on larval behaviour under *Ptth* KO). While the industry doesn't currently utilise any of these modifications on a large-scale basis, the future use of some genetic modifications could represent a serious welfare concern for farmed BSF. Future empirical work should determine the welfare impacts of these modifications before they are applied at scale, by studying alterations to behaviour or survival, as well as stress hormone levels.

Finally, selective breeding for particular life-history traits (such as fast development, or high fecundity) may result in genetic bottlenecks, inbreeding depression, and potentially colony collapse due to reductions in stress resistance and longevity (Gilchrist *et al.,* 2012); colonies should be carefully managed to maintain genetic diversity, and thus healthier populations (Hoffman *et al.,* 2021; Rhode *et al.,* 2020). Selective breeding has caused extensive welfare problems for many domesticated vertebrates (e.g. Paxton *et al.,* 2013); the BSF industry should view this as a cautionary tale, and take care to avoid similar concerns.

Crowding and injury

BSF larvae aggregate, which can cause the accumulation of waste (reducing growth rate), increase energy expenditure (due to intraspecific interactions), present thermoregulatory challenges, and promote competition for resources (although larvae may also be positively benefited by nearby conspecifics sharing their gut microbiota, as in house flies; Zhao *et al.* (2017)). Larval crowding can increase risks of illness by facilitating disease spread (Steinhaus, 1958), and potentially increase risk of injury. Reduced densities of 1-2 larvae per cm^2 of substrate were found to improve larval survival (Dzepe *et al.,* 2020).

BSF larval bodies are soft, making them susceptible to injuries caused by sharp objects. Injury may not kill a larva, but can dramatically increase infection risks – injured BSF are more susceptible to entomopathogenic nematode infection (with increased mortality resulting from infection; Tourtois *et al.* (2017)) and injured larvae have increased immune system activity following challenge with non-sterile environments (Zdybicka-Barabas *et al.,* 2017). Injury may be caused by improperly prepared substrates containing sharp material, inappropriate handling, or cannibalism. Although larvae are not typically reported to be cannibalistic under normal conditions (Caruso *et al.,* 2013), stressed larvae of other species (e.g. *Zophobas morio* (Coleoptera: Tenebrionidae), house flies) resort to cannibalism under adverse conditions; in *Z. morio* this is also a mechanism for disease spread (Lam *et al.,* 2007; Maciel-Vergara *et al.,* 2018).

BSF larval cannibalism may also be more common than anecdotally reported. Larval cannibalism of emerging adults has been recorded (Furman *et al.,* 1959), as well as consumption of dead and live larvae/pupae, particularly when reared in high density. Late-stage larvae given access to limited food were more likely to cannibalise other larvae than those given access to no food (47 vs 7%; Nguyen, 2010). However, Nguyen (2010) notes that in starvation conditions the risk of being injured while cannibalising other larvae is higher; thus, individuals might only cannibalise younger or smaller individuals in more typically size-heterogenous populations (not tested under their study design). Reduced localised access to nutritional resources may also promote cannibalism, explaining why survival rates are reduced in higher-density larval conditions (Barragan-Fonseca *et al.,* 2018; Dzepe *et al.,* 2020; Paz *et al.,* 2015).

Cannibalism of adults by larvae seems common (reviewed in Nguyen, 2010; Barrett, pers. comm.), so while mixed-housing methods may make repopulation easier (Fisher and Romano, 2020), BSF adults should be kept separate from larvae in industrial settings.

Handling-associated stress

Larvae are subjected to handling and disturbance, which may cause injury or stress (particularly if photophobic larvae are exposed to light). Larvae may be handled during the industrial rearing process to:

- change their substrate (removing frass, adding rigid materials, churning to change moisture and oxygen levels throughout the substrate);
- check health and development;
- harvest at smaller body sizes (e.g. for exotic pets);
- 'pre-processing', where live insects are cleaned prior to slaughter (see subsection 'pre-processing feed withdrawal periods and washing').

Some handling likely cannot be avoided while managing substrates and larval growth. More research is needed about how/if disturbance stresses BSF larvae but, given that most organisms react negatively to handling, it is safe to assume that handling may cause larvae some degree of discomfort (Baumann, 2019). Stress may be reduced by limiting the frequency/magnitude of disturbance and minimising larval exposure to light (by disturbing only in dark rooms). For example, producers might use self-harvesting systems (for harvesting late-stage larvae) or using batches of similarly aged neonates that can be harvested at the same time (if harvesting at multiple time points during development) to reduce the frequency of disturbance.

BSF adults are also likely to be stressed by handling or human activity: interruptions (apparently caused by movement or noise) can even cause mating to cease, and producers recommend keeping BSF adults in out-of-the-way locations to avoid disturbance (Yang, 2017a). Ethyl acetate is used to subdue adults for weighing (Dortsman *et al.,* 2017) but no data are currently available on the welfare impacts of ethyl acetate anaesthesia (see subsection 'Use of anaesthetics prior to slaughter').

3. Welfare during slaughter

Currently, no data are available on the welfare impacts of different slaughter methods for insects, and no welfare regulations exist to guide farmers in determining humane slaughter standard operating procedures (SOPs, Bear, 2019; Delvendahl *et al.,* 2022). To truly understand which slaughter methods are most humane, species-specific empirical studies are needed (De Goede *et al.,* 2013). In the absence of better data, we make a rough, categorical ranking of slaughter methods used for BSF larvae by considering which methods are more likely to be instantaneous (within one second) as rapid death reduces the risk of pain (IPIFF, 2019). Notably, many of the conclusions below for BSF larvae will apply broadly to all insects; however, the time-to-death of many methods will vary based on the organism's morphology or physiology, and therefore we limit our conclusions to BSF larvae.

More humane slaughter methods

Boiling/blanching, freezing in liquid nitrogen, and grinding are likely to be the most humane slaughter methods based solely on time-to-death (see also, Hakman *et al.,* 2013).

Boiling results in high body temperatures, damaging numerous molecular/cellular structures (enzyme denaturation, cell membrane disruptions, changes in pH, etc. all dependent on duration of exposure), leading to swift, organism-wide functional failures (Neven, 2000). High-heat methods induce 'heat coma' before death, where spreading depolarisation will silence central nervous system (CNS) function prior to mortality (Jørgensen *et al.,* 2020). All high-heat methods like boiling may cause a substantial amount of pain: fruit fly larvae are capable of thermal and mechanical nociception using class IV dendritic arborisation sensory neurons tiled across the body surface (Grueber *et al.,* 2002; Neely *et al.,* 2011), and exhibit protective behavioural responses to high temperatures (Chattopadhyay *et al.,* 2012; Oswald *et al.,* 2011; Petersen *et al.,* 2018). However, pain should be very brief as the transfer of heat from boiling water (100 °C) to a single larvae should result in near-instantaneous nervous system inactivation.

Boiling SOPs may affect the rapidity of death, particularly when insects are killed in large batches. For example, producers may use lower water temperatures in order to save energy in support of sustainability goals, but more tepid water may not kill larvae instantly. If a large number of larvae are placed in the water at once, they may drop the temperature of the water so that death is not instantaneous. In addition to rapidly causing death, this method has been shown to minimise lipid oxidation, microbial contamination, and colour alteration while initiating dehydration (Larouche *et al.,* 2019; Sindermann *et al.*, 2021; Zhen *et al.,* 2020), suggesting it may be a good method for product quality as well as welfare purposes (with the right SOP).

Similar to boiling water, larvae immersed in liquid nitrogen (LN_2; -196 °C) will chill rapidly and death is likely to be near-instantaneous. Body water inside LN_2-treated insects will freeze, causing mechanical damage to cells, and direct chilling injuries will result from the cold shock (again, resulting in protein denaturation and cell membrane disruptions; Drobnis *et al.* (1993), Muldrew *et al.* (2004), Privalov (1990)). Freezing larvae in LN_2 presents similar SOP issues to boiling: the number of larvae, LN_2 temperature, ratio of larvae: LN_2, and shape of batches of larvae when immersed in LN_2, should all be carefully managed to ensure instant death. LN_2 deaths may cause brief, significant pain; *Drosophila* larvae are capable of cold nociception using class II and III multidendritic and chordotonal neurons (Turner *et al.,* 2016, 2018), but again the nervous system should be inactivated rapidly in LN_2.

Shredding kills insects through mechanical disruption of their tissues and cells. Little information is available on grinding/shredding methodologies, but some producers have reported that shredding is probably the most humane method as death occurs in less than a second (Erens *et al.,* 2012; though there may be handling-associated stress for larvae being shaken in the grinder prior to reaching the shredding apparatus). Given the design of many of these machines, incomplete shredding or prolonged deaths appear unlikely, but this should be empirically tested. *Drosophila* larvae exhibit protective behavioural responses to mechanical nociceptive stimuli (Lopez-Bellido and Galko, 2020). However, shredding is likely to sever connections between peripheral and central nervous systems almost immediately, preventing significant pain.

All three of these methods are also likely to be most humane for BSF adults, which may be slaughtered prior to restocking mating cages. Because of their capacity for flight, adults may try to 'escape' particular slaughter methods, resulting in slower and less humane death (e.g. by flying away from the surface of boiling water, drawing out their deaths to several seconds). Anaesthetics like isoflurane (see subsection 'use of anaesthetics during slaughter') should be employed for adults prior to employing one of these 'more humane' methods of slaughter, in order to maximise the rapidity and humaneness of adult death.

Less humane slaughter methods

Sand roasting, microwaving, sun baking, oven baking, freezing in air, and asphyxiation are likely to be less humane slaughter methods for BSF larvae based on time-to-death.

When sand roasting is used, sand is heated in a pan or rotating drum to >150-200 °C prior to the addition of larvae in a 1:1 or 2:3 larvae-to-sand ratio (EAWAG, n.d.). Conductive heat transfer between larvae and the sand should raise larvae to lethal temperatures very quickly, once fully immersed. However, both rotating drums and pans may not immerse all larvae entirely within the sand instantly, limiting conductive heat transfer to many larvae in the first few seconds of exposure. Convective heat transfer from hot air above the sand surface will heat larvae more slowly. Rotating drums are more likely to be instantaneous: the motion of the drum should quickly bring all larvae fully into contact with the sand if larvae are added while the drum is in motion. When pans are used, sand should be continuously stirred as larvae are added so that larvae do not pile up on the sand surface but instead are quickly and completely immersed in sand to generate higher conductive heat transfer. A lower larvae-to-sand ratio is more likely to immerse each larva completely, as well as avoiding substantial reductions in pan/drum temperature via the introduction of cooler material.

Microwaving insects will kill them by dielectric heating (described in Yadav *et al.* (2014)). Microwaving appears to be relatively common for BSF larvae; machines can process more than 4,000 W/kg of fresh larvae (dependent on SOP and microwave power) and are typically 2,450 MHz. The process of completely drying out larvae takes 6-15 minutes (EAWAG, n.d.; MAX Industrial Microwaves, 2016, n.d), but death will occur sooner, as larvae reach CT_{max}. A 500 W microwave source killed 100% of smaller-bodied red flour beetle (*Tribolium castaneum,* Coleoptera: Tenebrionidae) larvae within 28 seconds (in a 50 gram sample of grain, so 10,000 W/kg of material; Vadivambal *et al.,* 2008). It is challenging to extrapolate from this dataset to industrial BSF slaughter scenarios (small sample size, variation in larval body size, and the obstructive substrate in Vadivambal *et al.,* 2008), but industrial microwaves are unlikely to slaughter all larvae instantaneously under most SOPs, due in part to the non-uniform distribution of microwave energy. Time-to-death can be hastened by increasing the W/kg of energy experienced by larvae and by placing larvae in a single layer relative to the energy source.

Industrial microwaves either have a conveyor belt that moves larvae along a track stationed underneath the microwave units or a small drum with a stir bar that mixes a shallow pool of larvae. Both of these methods serve to move larvae around in relation to the microwave source, as microwaves traditionally have problems with uneven distribution of energy and low substrate penetration (Enviroflight, n.d.; Hossan *et al.,* 2010; Yadav *et al.,* 2014). If larvae are not continuously moved, or are layered, microwave units will kill larvae at very different rates depending on their placement in relation to the energy source. Notably, moving larvae (for example with a stirring apparatus) could also cause additional stress prior to mortality.

Sun baking will take several hours, depending on ambient temperature, relative humidity, and solar intensity, to kill BSF larvae either through desiccation or overheating. In addition, BSF larvae are photophobic (Canary *et al.,* 2009; Newton, n.d.), and will attempt to move away from the sunlight during this method of slaughter, suggesting that sunlight likely induces additional stress (Mat *et al.,* 2021). There is no SOP that will make sun baking instantaneous or humane.

Ovens are typically heated to 60-65 °C (EAWAG, n.d.; Larouche *et al.,* 2019). Both the lower air temperatures in oven baking (as compared to sand roasting or blanching), and slower transfer of heat from the air to the larvae, suggest that this method will not be instantaneous and could take many seconds to a few minutes to kill all larvae, depending on SOP.

Time-to-death using this method can be reduced by: (1) increasing temperatures and air speeds within the oven; (2) placing larvae on pre-heated trays before placing them in the oven (increasing conductive heat transfer and avoiding the introduction of cooler pans, which will lower the temperature of the oven and keep the larvae touching the tray from heating as quickly); (3) maximising larval contact with heated air by laying larvae flat and avoiding clumps on heating trays; or, (4) introducing smaller amounts of larval mass per unit of heated air. However, SOPs using this method are unlikely to ever achieve instantaneous slaughter.

Freezing in air has been considered, anecdotally (Bear, 2019; Erens *et al.,* 2012), a humane method of insect euthanasia because it is thought to anaesthetise the insect prior to death by gradually chilling them. However cold does not have any analgesic effects and is not considered humane as an anaesthetic for invertebrates according to veterinary practitioners (AVMA, 2020; Cooper, 2001; Gunkel and Lewbart, 2007; Pellett *et al.*, 2013). Murray (2012) even recommends the use of an inhalation anaesthetic prior to any freezing-based slaughter methods.

Gradually chilling insects causes a reduction of metabolic rate and the onset of a (reversible) 'chill coma' (e.g. Andersen *et al.*, 2018; Tutun *et al.*, 2020) which occur prior to death. Chill coma is believed to result from loss of Na^+/K^+ ATPase function, causing an equilibration of ions across the membrane and thus reduced excitation in muscle cells, with the spreading depolarisation shutting down the central nervous system (CNS; Andersen *et al.*, 2018; Goller and Esch, 1990; Hosler *et al.*, 2000). CNS function will cease before mortality is achieved, but even CNS failure will take several seconds to minutes (depending on freezer air temperature) and this period of time may involve significant pain associated with nociceptive cold.

Freezers used to chill insects may vary in temperature from -80 to 4 °C, though Larouche *et al.* (2019) report a temperature of -20 °C or -40 °C for killing BSF larvae. No data currently exist for BSF larval time-to-death (or immobility), but as a tropical species it is probable that their cold tolerance is quite poor. Data from adult honey bees (*Apis mellifera,* Hymenoptera: Apidae) shows movement for up to a minute at -80 °C, with only 76% dead after 10 minutes at -20 °C (Tutun *et al.*, 2020). While adult honey bees and larval BSF are likely to be different in their response to cool temperatures, a reasonable induction from the honeybee data is that freezing in air is unlikely to ever be instantaneous for BSF larvae. Similar changes to SOP as listed in the oven baking section (pre-cooling trays, etc.) could be used to reduce time-to-death.

Insect asphyxiation times depend on life stage, body size, metabolic rate, environmental conditions (temperature, humidity, gas composition of the air) and other adaptations to hypoxia that specific insect species may have developed. Some gasses used to suffocate insects may also be used to anaesthetise insects – for example CO_2, which has been shown to rapidly block signal transduction at the neuromuscular junction (Badre *et al.*, 2005). However, there is doubt among veterinary specialists that CO_2 has any analgesic effects (Cooper, 2011; Gunkel and Lewbart, 2007), and it is not recommended as a humane method of euthanasia for insects in Murray (2012). More studies on the impact of CO_2, N_2, and other gasses will be needed to understand the welfare impacts of their use in asphyxiating slaughter methods.

Insect death by suffocation is generally slow (many hours). The larvae/nymph stages of 12 different insect museum pests took between 3 and 144 hours to achieve 100% mortality (Rust and Kennedy, 1993). Hypoxic conditions induce hyperventilation and loss of spiracular control, significantly increasing water loss and causing desiccation. In addition, anaerobic respiration may generate an accumulation of metabolic by-products that increase the acidity of the insect haemolymph (Maekawa and Elert, 2003; Schimpf *et al.,*, 2012). Nitrogen is commonly used for insect asphyxiation, but using CO_2 can increase the rapidity of mortality. In addition, increased temperatures (40 °C; Valentin 1993), reduced oxygen availability (Soderstrom *et al.,* 1992), and lower relative humidity (30% or less; Rust and Kennedy 1995) can decrease time-to-death.

Use of anaesthetics prior to slaughter

Slaughter might also be made more humane by rendering insects unconscious in a non-stressful way prior to death, through the use of anaesthetics. Veterinary associations recommend anaesthetics be applied to insects prior to painful stimuli (AVMA, 2020). Cold, CO_2, FlyNap (Triethylamine and alcohols), ethyl acetate, and halogenated ethers (sevoflurane, halothane, isoflurane) are all anaesthetics reportedly used for insects in other contexts (research and educational settings, zoos/aquariums, and veterinary practices; Cooper (2011), MacMillan *et al.* (2017), McCallion *et al.* (2021)). No studies have tested which method of anaesthesia is most humane for any insect species. Cold and CO_2 have been shown to have adverse follow-up effects on behaviour and physiology for at least a day post recovery, especially when compared to halogenated ethers (Gooley and Gooley, in press; MacMillan *et al.,* 2017). Some veterinary specialists suggest that both cold and CO_2 may have limited analgesic effects for insects, are less likely to be humane, and should be discontinued or used in a limited capacity (e.g. surface handling only; Cooper, 2001, 2011). Inhalation anaesthetics and CO_2 can be stressful or physiologically harmful for vertebrates, especially if given incorrect doses (Marquardt *et al.,* 2018; Turner *et al.,* 2020).

The use of anaesthetics prior to slaughter does not appear to be a standard practice in the BSF industry (but see Bear, 2019). Zhen *et al.* (2020) used CO_2 to render BSF unconscious prior to slaughter and found no significant effects on product quality in larvae blanched following CO_2 treatment, however they did not assess the impacts of CO_2 on welfare. More research will be needed to determine a standard operating procedure for anaesthetic use in insects prior to slaughter. Future studies should ascertain: (1) the physiological and behavioural effects of different dosages of different inhalation anaesthetics; (2) the time-to-unconsciousness of these dosages; and (3) the length of time these anaesthetics render the insect unconscious while undergoing different methods of slaughter; and (4) any effects of these anaesthetics on the final product safety or quality using different slaughter methods.

Pre-processing feed withdrawal periods and washing

Some producers starve, or 'fast', insects prior to slaughter for 1-2 days in order to empty the insects' guts (Van Huis, 2021), though how common this feed withdrawal practice is in the BSF industry is unclear (Larouche, 2019). However, in some insects 'fasting' can induce cannibalism (e.g. crickets, reported in Bear (2021)), suggesting insects are still experiencing a need to feed during this period of starvation. In addition, dehydration stress can be severe for starved insects (Schimpf *et al.*, 2012), and BSFL starved for 96 hours saw significant reductions in moisture content (Larouche, 2019) which may put them at risk of dying from desiccation. For BSFL, 84 hours was required for 99% of larvae to have evacuated their guts (median = 72 hours), much longer than the 1-2 days often reportedly used in the industry. In addition, this period of starvation does not appear to be necessary for product safety purposes, as microbial load was not affected by starvation in mealworms or BSFL (Larouche, 2019; Wynants *et al.,* 2017).

In addition, some producers may 'wash' larvae by spraying them with water or dipping them in agitated water prior to slaughter (Larouche, 2019). Although generally quite short, this handling method could cause stress by immersing larvae in an unusually high-moisture environment (BSFL generally live in a semi-aqueous, but not 100% moisture, and low oxygen environment; see sections on 'moisture' and 'oxygen') and physically agitating them. No studies have investigated the effects of washing BSFL on microbial contamination, but washing mealworm larvae produced no product safety benefit (Wynants *et al.,* 2017); however, it is possible the different substrates used to rear BSFL may impact the effect of washing on microbial contamination, and this should be empirically investigated.

4. Areas of concern and recommendations

From this review, we have determined several areas of current (Table 2 and 3) and future concern (Table 4) for farmed BSF welfare. We make recommendations for reducing suffering/promoting positive experiences in accordance with the five freedoms model of animal welfare; notably, these recommendations are hampered by the limited data on BSF biology and welfare and future empirical work could alter best practices and recommendations given here.

5. Urgent future research into black soldier fly welfare

More work on insect sentience, and thus capacity for welfare, would be valuable for researchers attempting to understand the potential for suffering in farmed insect systems. Particularly, work that addresses how we might assess pain, discomfort, fear, or distress in insects will be essential to best use the five freedoms framework to assess insect welfare. Significant additional empirical research is needed, that will study BSF with welfare (and not simply growth or survival) in mind, including:

- Studies of adult mating, ovipositing, and foraging behaviours in natural vs farmed settings, to better understand the impacts of captivity on adult behaviour.
- Studies that address the impacts of cage densities on the behaviour and welfare of adults.
- Determining the nutritional preferences of adults, and the impacts of different diets on longevity, behaviour, reproduction, and welfare.
- More controlled studies of the welfare impacts of abiotic variables, nutrition, and their interactive effects on larvae, at the scale typically used by the industry (or expected to be used as the industry grows).
- Controlled studies on population-level variance in the impacts of abiotic conditions or nutrition.
- Studies that determine sublethal impacts of handling and abiotic factors on welfare (e.g. looking at stress hormone levels).
- Studies that identify probable parasites/pathogens associated with BSF and their welfare impacts, particularly prior to epizootic disease outbreaks.
- Studies that address the impacts of low-nutrient-content substrates on lethal and sublethal larval welfare at different rearing scales (e.g. the effect of feeding manure in Miranda *et al.* (2020) vs Myers *et al.* (2008) and Lalander *et al.* (2019)).
- Studies that assess the effectiveness of starvation/washing pre-slaughter procedures in reducing product microbial contamination levels of BSFL reared on different substrates (such as manure). If these pre-slaughter procedures are determined to be effective in any particular case, these studies may assess develop guidelines to minimise larval stress.
- Studies that determine the pre-slaughter stress caused by each slaughter method, as well as pain experienced during slaughter, and the time each method takes to kill (or knock unconscious) all individual organisms, in order to develop humane SOPs.
- Studies that determine the most humane methods of rendering insects unconscious (e.g. anaesthetics), which may reduce larval suffering prior to death. In addition, the impacts of these processes on the final product should be determined.

6. Conclusions

Insect welfare is an emerging field of great interest for academics, producers, and consumers (Bear, 2019; De Goede *et al.,* 2013; Delvendahl *et al.,* 2022; Erens *et al.,* 2012; IPIFF, 2019). To rear insects in high-welfare conditions, substantive reviews are needed that incorporate a species-specific natural history, information from producers, and scientific data on physiology, morphology, behaviour, and

Table 2. Areas of greatest current concern for farmed black soldier fly (BSF) welfare, and recommendations for welfare improvements based on current data.

Concern	Recommendations
Starvation & denial of feeding behaviour	Adult BSF may prefer honey to sugar water; all adults should be provided food in feeders that prevent drowning.
Unmet needs for natural mating behaviours	BSF adults should be provided with more space, and lower rearing densities, to encourage natural mating behaviours. We recommend a minimum of 3 m^3 of space, with 100 or fewer adults, at this time.
Unlit cages following eclosion	Adults kept in the dark for 2-10 days following eclosion experience lethargy and delay their natural mating sequences. BSF should be kept on a natural day-night cycle following eclosion.
Non-instantaneous slaughter, without anaesthesia	Instantaneous slaughter methods should be preferentially used, particularly grinding, boiling, and freezing in $LN_{2,}$ with SOPs that ensure humane death for all insects in a batch. Inhalation anaesthetics recommended by vets (e.g. isoflurane) should be used prior to slaughter (after ensuring no harmful effects of anaesthetic use on consumers of BSF products).
Pre-slaughter starvation	96 hours of starvation does not reduce BSFL microbial contamination and should be discontinued (though see future research direction #9).
Moisture/oxygen-induced larval stress and mortality	A moisture level between 50-70% is ideal across substrate types, but should be experimentally determined for each new substrate (influenced by texture and composition). Substrates should have a maximum depth of 5 cm, to prevent anaerobic conditions deeper in the substrate. Forced aeration, manually churning substrates, and the addition of high-rigidity, porous substrates can all increase oxygen levels.
Exposure of photophobic larvae to light & handling-associated stress	Larvae should only be disturbed in dark rooms. Handling-associated stress and disturbance can be minimised by allowing larvae to self-harvest, or rearing larvae in age-matched batches that can be harvested all at the same time. Pre-slaughter washing has no effect on microbial contamination in other insect larvae (more data are needed on BSFL reared on different substrates). Producers may consider dropping the practice altogether, or reducing potential stress factors by using a shorter, gentler 'dip' method over sprays or agitation.
Lack of pupation substrate for post-feeding larvae	Larvae should be provided with appropriate pupation substrates (wood shavings appear optimal) while awaiting slaughter or consumption, including when being shipped live to consumers, in order to permit natural burying behaviours.
Nutritional inadequacies for larvae	Brewer's grain, certain kinds of vegetable/fruit/nut/grain wastes, and animal faeces may all be homogenous and low in nutrients. Maintaining at least 50% nutrient content in any substrate is recommended. Producers should consider using heterogeneous substrates, potentially by mixing numerous waste streams (or adding glucose, plant oils, proteins, etc.). Substrates with large amounts of non-digestible material should be inoculated with beneficial microbes to aid digestion.

Table 3. Areas of lesser current concern for farmed black soldier fly (BSF) welfare and recommendations for welfare improvements based on current data.

Concern	Recommendations
Cannibalism of adults	BSF adults and larvae should always be reared separately.
Crowding-associated larval injury and death	Larvae should be provided with plentiful nutrition and maintained at densities of 1-2 larvae/cm^2, to discourage cannibalism, overheating, competition, and pathogen transmission.
Rearing and slaughter of BSF shipped to consumers	Explanation: Live-shipped larvae are generally kept in suboptimal conditions in order to prevent pupation, including: no access to food/water, no pupation substrates, cool temperatures. In addition, consumers are told to dispose of excess larvae by freezing them for 24 hours. Recommendation: Producers should provide food, pupation substrates, and guides to ideal rearing conditions; they should recommend boiling water to kill larvae in small batches, as a faster death may reduce suffering. Producers should consider allowing smaller amounts of larvae to be purchased more frequently, to avoid poor rearing and slaughter conditions by unpractised consumers.

Table 4. Areas of emerging concern for farmed black soldier fly (BSF) welfare and recommendations.

Concern	Recommendations
Pathogens and parasites	Producers should work closely with labs that can diagnose and study any diseases/parasites identified in their facilities. Researchers should survey wild BSF for pathogens and parasites. In addition, producers should: (1) clean production facilities regularly; (2) sterilise equipment whenever it contacts a new batch of insects; (3) only allowing trained staff, with properly sterilised personal protective equipment, into production facilities; (4) monitoring populations vigilantly for signs of disease and quarantining poorly performing populations immediately; (5) preventing unintentional introduction of wild dipterans to rearing stocks; (6) avoiding overfeeding (no more than 30-50% of provided food left undigested); (7) manipulating microbial communities within substrates brought in for larval rearing (e.g. enhance beneficial/sterilise harmful microbes); (8) separating adult/larval rearing; and (9) separating rearing facilities for different insect species to reduce interspecific transmission.
Genetic modification of adults and larvae	The impact of any new genetic modifications, or selective breeding programs, on BSF welfare should be studied proactively. For example, flightless adults violate the species' ability to exhibit normal flight behaviours. Larval modifications for increased feeding time and body size may also negatively impact welfare or constrict normal behaviours.
Feeding of animal faeces to larvae	Animal faeces tend to be homogenous, low nutrient content substrates that reduce larval survival and decrease body sizes; they should not be fed to larvae before processing methods (e.g. microbial inoculation, mixing with other wastes) can be developed that make them suitable for larval survival and development.

welfare (where such data exists). These reviews should be continually updated as industry practices/technology progress and our understanding of the species' response to farmed conditions deepens. This paper is a first step toward this goal, as it provides the first comprehensive review of BSF welfare in the context of our knowledge of basic BSF biology and current industry practices.

Insects often have a negative reputation, particularly those viewed as 'pests' (a label once applied to BSF; Tomberlin and Van Huis (2020)), which may lead to reduced empathy for their interests. In addition, the differences between the anatomy and behaviour of vertebrates and insects can foster a sense of 'ethical distance' (Bear, 2021), which may reduce our sense of urgency to investigate their welfare at the individual level. Nevertheless, many producers care about their insect livestock and need guidance about how best to raise and slaughter them (Bear, 2019, 2021) – guidance that is especially important given the trillions of insects affected as the food and feed industry continues to grow (Rowe, 2020; Schukraft, 2019). It is no surprise, therefore, that there have been many calls for more research in this area (e.g. IPIFF, 2019), all of which we echo. However, that research will not happen in time for many producers – and untold numbers of insects – unless more resources are directed toward extending welfare and sentience research to these animals. Collaborations between funders, producers, welfare ethicists, and entomologists will be essential to providing timely, species-specific, insect-relevant welfare guidance for the insects as food and feed industry.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2022.0041

Figure S1. BSF pupae and dermestid beetle larvae that arrived together in one shipment from a producer to Barrett in 2021, with both a fungal infection (A: on debris; C: on BSF pupae) and mites (B: on dermestid beetle larvae; D: on BSF pupae).

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Conflict of interest

Meghan Barrett reports financial support was provided by Rethink Priorities. Meghan Barrett and Bob Fischer report a relationship with Rethink Priorities that includes:

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