

Research Article

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Grinding as a slaughter method for farmed black soldier fly (*Hermetia illucens*) larvae: Empirical recommendations to achieve instantaneous killing

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Abstract

At least 200 billion black soldier fly (*Hermetia illucens*) larvae (BSFL) are reared each year as food and feed, and the insect farming industry is projected to grow rapidly. Despite interest by consumers, producers, and legislators, no empirical evidence exists to guide producers in practicing humane – or instantaneous – slaughter for these novel mini-livestock. BSFL may be slaughtered via freezing, boiling, grinding, or other methods; however standard operating procedures (SOPs) and equipment design may affect the likelihood of instantaneous death using these methods. We tested how larval body size and particle size plate hole diameter affect the likelihood of instantaneous death for black soldier fly larvae that are slaughtered using a standard meat grinder. Larval body size did not affect the likelihood of instantaneous death for larvae that are 106–175 mg in mass. However, particle size plate hole diameter had a significant effect on the likelihood of instantaneous death, with only 54% of larvae experiencing an instant death when using the largest particle size plate (12-mm hole diameter) compared to 84% using the smallest particle size plate (2.55 mm). However, a higher percentage of instantaneous death (up to 99%) could be achieved by reducing the proportion of larvae that become stuck in the machine. We conclude by outlining specific recommendations to support producers in achieving a 99% instantaneous death rate through specific SOPs to be used with similarly designed machines. We also develop a protocol for producers that wish to test their own grinding SOPs.

Introduction

The insects as food and feed industry (IAFF) is the single, largest animal livestock farming initiative in human history, already rearing over 1.2 trillion insects a year (Rowe 2020). The IAFF industry has the potential to help achieve sustainable agriculture goals by optimising, and possibly minimising, land and other resources used to obtain animal protein while promoting local circular food systems that turn waste into food, feed, and fuel (van Huis & Tomberlin 2017; Parodi *et al.* 2022; Purkayastha & Sarkar 2022; van Huis & Gasco 2023). These benefits can result in greater economic and food security (van Huis 2013; Chia *et al.* 2019), especially crucial at a time when the global human population is growing (United Nations 2022) and climate change is heightening food insecurity (Makkar 2018).

The IAFF industry is growing rapidly to meet the demand of emerging markets (de Jong & Nikolik 2021; World Wildlife Fund & Tesco 2021); for instance, Makkar *et al.* (2014) estimate that up to 100% of fishmeal could safely be replaced with insect protein in aquaculture feed which would result in 160–320 trillion additional insects reared each year (dependent on body mass; Rowe 2020). Insect protein is also suitable for human, vertebrate livestock (chickens, pigs, etc), and pet consumption markets that represent additional insects reared over and above that estimate (de Jong & Nikolik 2021). All this demand means that large facilities are currently being built and planned that could ultimately rear three trillion individual insects or more each, every year (van Huis & Tomberlin 2017; Innovafeed 2020).

As the industry grows, interest in the welfare of insects as mini-livestock has also emerged from consumers, producers, academics, legislators, and advocacy groups for the industry and animals (de Goede *et al.* 2013; Bear 2019; IPIFF 2019; Lambert *et al.* 2021; van Huis 2021; Delvendahl *et al.* 2022; Barrett *et al.* 2023a,b; DEFRA 2023; Eurogroup for Animals 2023; Klobučar & Fisher 2023; Kortsmits *et al.* 2023; Voulgari-Kokota *et al.* 2023). Sentience is generally considered a precondition for the consideration of welfare (though see Dawkins 2017) and there is currently no scientific consensus on insect sentience (Adamo 2016, 2019; Barron & Klein 2016;

Klein & Barron 2016; Gibbons & Sarlak 2020; Mikhalevich & Powell 2020; Lambert *et al.* 2021; Loon & Bovenkerk 2021; Overgaard 2021; Kakrada & Colombo 2022; Elwood 2023; Voulgari-Kokota *et al.* 2023; Barrett & Fischer 2024).

However, a recent review of neurobiological and behavioural research on insects indicates sentience is plausible (Gibbons *et al.* 2022), especially in some orders with farmed insects (e.g. black soldier flies [*Hermetia illucens*], BSF, in the order Diptera). Importantly, developmental stage may impact the likelihood of sentience in an insect species, especially in the holometabolous insects that undergo a complete metamorphosis (for discussions, see Gibbons *et al.* 2022; Barrett & Fischer 2023; Fischer & Sandall 2023). For instance, Gibbons *et al.* (2022) found that adult dipterans meet six of eight criteria for sentience according to the Birch *et al.* (2021) invertebrate sentience framework while slaughter-age larval dipterans met only four of eight; the remaining two, or four, criteria, respectively, had not yet been assessed for that order at that life stage. While the framework rated adult dipterans as having “strong evidence for sentience”, last instar larval dipterans still fulfilled enough criteria for there to be “substantial evidence for sentience” at this life stage. Therefore, given the scale of the IAFF industry, these data suggest it to be prudent to begin to protect farmed insect welfare from a precautionary perspective, even without complete information on insect sentience at all life stages (Birch 2017; Barrett & Fischer 2023).

Of particular interest to producers (Bear 2019), legislators (UK Government 2015; Council of Animal Affairs 2018), and the media (Sebo & Schukraft 2021; Carlson 2023; Reynolds 2023) has been the welfare of insects during slaughter. Accordingly, the lack of guidance on best practices for humane slaughter of insects represents a significant risk to the industry’s social licence to operate (Barrett & Adcock 2023). This is especially important given that the industry’s most informed and youngest consumers appear to be the most concerned about insect pain in the industry (at least in the US; Fukuda *et al.* 2023), suggesting that – alongside evidentiary trends (Gibbons *et al.* 2022) – social trends are unlikely to make the issue of insect welfare at slaughter less salient to the public over time.

BSF are expected to become the most farmed insect species in the near future. BSF are generally reared in batches and slaughtered as larvae (BSFL). BSFL may be slaughtered using a variety of different methods, including (but not limited to): freezing (in air or liquid nitrogen); baking in a convection oven; roasting in sand or sunshine; microwaving; boiling/blanching; asphyxiation; and grinding/shredding (for a review, see Barrett *et al.* 2023a). Humane slaughter may be defined as “when an animal is either killed instantly or rendered insensible until death ensues, without pain, suffering or distress” (Royal Society for the Prevention of Cruelty to Animals [RSPCA] 2023). Clearly, only some of these methods are likely to satisfy this standard. Importantly, the methods and conditions that generate the most humane slaughter process for BSFL may be different than for previously studied species (mostly vertebrates) due to the number of animals to be killed and their unique physiology/morphology (e.g. small bodied, ectothermic, etc). Barrett *et al.* (2023a) suggest boiling/blanching, freezing in liquid nitrogen, and grinding/shredding are the methods most likely to be instantaneous, and thus humane, for BSFL; however, they acknowledge that standard operating procedures (SOPs) are needed to define what practices achieve humane death using each method while remaining practical and economical.

We set out to test if grinding can result in instantaneous (< 1 s), and thus humane, death for BSFL. As larval body size at time of

slaughter can vary, we tested two different slaughter-relevant larval body sizes. Producers may also vary the grind consistency of the larval product by changing the hole diameter of the particle size plate on the grinder; therefore, we also tested the effect of particle size plate hole diameter on the likelihood of instantaneous death. From these data, we develop a set of practical recommendations for producers on how to generate grinding SOPs with the greatest likelihood of instantaneous death. Further, given significant variance in machinery design from our own grinder, we provide a protocol for producers interested in testing their own machinery and SOPs for BSFL at four different body sizes.

Materials and methods

Larval rearing

BSF were obtained from a colony that is maintained at the Forensic Laboratory for Investigative Entomological Sciences Facility at Texas A&M University, USA. The colony originated from a colony in Tifton, GA, USA, and the adult colony is maintained and eggs were collected according to methods described by Miranda *et al.* (2020). Upon eclosion (i.e. the act of emerging from an egg case), 27 replicates of 0.5 g of newly hatched larvae (approximately 10,000 larvae) were weighed on an Ohaus® Adventure™ Pro AV64 balance (Ohaus® Corporation, Pine Brook, NJ, USA) and each replicate was placed on 500 g of Gainesville diet (50% wheat bran, 30% alfalfa meal, and 20% corn meal; Hogsette 1992) saturated with RO (reverse osmosis) water (70%) in 0.5-L deli food storage containers (Amazon.com Inc, Seattle, WA, USA). The newly hatched larvae were allowed to feed for approximately one week and then the contents (i.e. larvae, diet, and frass [i.e. insect waste]) of the 0.5-L containers were poured on top of 8 kg of Gainesville diet (70% moisture) placed in the centre of a 30-L Sterilite® ClearView Latch™ storage container (Sterilite®, Townsend, MA, USA), with approximately 800 g of dry diet placed around the perimeter of the wet diet to prevent developing larvae from escaping. To obtain medium size larvae, two of the 0.5-L containers with seven day-old-larvae (DOL) were placed in one pan with 8 kg of diet (approximately 20,000 larvae per pan), while to obtain large larvae, only one container with 7 DOL (10,000 larvae per pan) was placed in each 30-L pan. In total, 18 pans were set up for medium-size larvae and nine pans for large larvae. The larvae were allowed to feed in the pans for one week and then sifted from the diet and frass for use in the experiments described below.

Proportion of instantaneous death due to mechanical separation of the body segments

We assessed the likelihood of instantaneous mortality after receiving a single cut to a particular body segment in the medium- and large-sized larvae (18–19 DOL), as well as 7 and 23–24 DOL. Each larva used was weighed individually to determine body weight and confirm no consistent differences in body size across treatments within a group.

Larva were placed individually on a hot plate kept at 32°C in room temperature air (23.1–24.3°C) and allowed to acclimate for 30 s prior to the beginning of the trial. The use of the hot plate prevented larval bodies from rapidly chilling due to a combination of convective cooling and haemolymph loss; larval bodies were found to drop 1–5°C within 5 s of administering a cut without using the hot plate, which influenced our ability to use movement to assess time-of-death. The hot plate kept larval bodies well above

thermal minima so that movement could always be performed and assessed (Addeo *et al.* 2022).

For this work, 130 larvae were used from each age/size group, with ten assigned to receive a single dorso-ventral horizontal cut, using a razor blade, in one randomly assigned body segment (S1–S12) and ten control larvae that underwent the same treatment programming but received no cut. One second after receiving the cut, both halves of the larvae were assessed for movement using a poke on the dorsal surface from an MT-29/1HT needle microprobe (Physitemp, Clifton, NJ, USA). Larvae that were ataxic when poked were considered ‘dead’. Movement was assessed to be ‘controlled’ (e.g. locomotion, circular movements of the head or posterior, thrashing, rolling, self-cannibalism, etc; see Supplementary Videos 1–10 included in Barrett *et al.* 2023c) or ‘uncontrolled’ (e.g. a twitch response). Only controlled movements were to be counted as ‘alive’, but no uncontrolled responses were exhibited outside our pilot trials, which occurred at a cooler temperature. The cessation of controlled behaviour is a frequent behavioural endpoint marking death in lethal studies of insects (e.g. Lighton & Turner 2004; Barrett *et al.* 2023d), though it has not yet been verified metabolically or with electrophysiology in this specific context. Complicating matters, ‘controlled’ behaviour has been observed in the headless portions of decapitated vertebrates (Michel 2019). Nevertheless, if precautionary considerations justify attempting to ensure that BSFL are slaughtered humanely, then these same considerations justify treating these behaviours as markers that the animals have not yet died.

In all cases, only one half of the body moved after receiving the cut: for medium-sized larvae, cuts in segments 1–5 indicated the posterior half of the larvae was alive, while cuts in segments 6–12 indicated the anterior half of the larvae was alive; for large-sized larvae, cuts in segments 1–4 indicated the posterior half of the larvae was alive, cuts in segments 5–12 indicated the anterior half of the larvae was alive.

Larvae were assessed for movement for an additional 90 s; if movement ceased, a poke from the probe was used to assess if the larva was alive-but-inactive or had ceased moving altogether and could be presumed dead. If dead, time of death was recorded. At 90 s, larvae were removed from the experimental arena and killed by placing a freezer.

Assessing instantaneous grinding SOPs at scale

To grind the larvae, we used a Univex MG89 Meat Grinder (Salem, NH, USA) with an internal rotating blade immediately anterior to the particle size plate. We tested three different particle size plates with holes 2.55 mm (small), 5.5 mm (medium), or 12 mm (large) in diameter, and two larval body sizes (medium- and large-sized larvae). We determined larval body size by weighing five groups of ten larvae from each batch (two batches of larvae at each size, used for two separate trials) to obtain an average larval weight.

We ran three replicates in each trial for each plate-larval size combination and two trials were performed on two separate days. For each replicate, we turned on the grinder and then poured 500 g of larvae into a funnel, inserted into the hopper of the grinder. We collected the mass of all the ground material that made it through the particle size plate into a plastic container. When larvae stopped passing through the particle size plate we stopped the grinder and collected the mass of all the material stuck behind the particle size plate at the internal rotating blade. We also collected and counted any larvae that were stuck in the tube leading to the

blade and noted any obvious injuries (e.g. open wounds or distortion of the body shape from pressure). We noted the time we started and stopped pouring the larvae in the hopper in addition to the time larvae started and stopped coming out of the particle size plate to calculate loading and output rates for each replicate. Between each replicate, we took apart and cleaned the entire machine prior to reassembly.

For each group of larvae (those that passed through the plate and those stuck at the blade), we carefully stirred the material before taking three 8–12 g subsamples from each, placing the subsamples in individual plastic vials filled with 70% EtOH; the remaining sample material was frozen for 24 h to kill any living larvae. We stored subsamples at -4°C until counting to prevent decomposition.

To count each subsample, we drained the EtOH using an ultra-fine mesh kitchen strainer and carefully spread out the remaining material onto a paper towel using forceps. Using the data from the trial on the mechanical separation of body segments in individual larva, we counted and recorded the cut location for any larval bodies that were intact enough to be ‘alive’ (see Figure A in Supplementary File 1). This method prevented double counting of bodies, as only one side of a separated larva was counted. When there was an incomplete separation of the larval body, or when separation occurred on a diagonal, we recorded the segment of the wound that reached the farthest across the midline of the animal (as this is closest to the nervous system’s position). We also counted any larvae that were uncut (e.g. whole).

For each replicate, we divided the mass of each group of larvae (those at the blade and that passed through the plate) by the average weight of an individual larva from that batch to determine the number of larvae in each group. We then added these numbers to the number of larvae stuck in the tube to get the total number of larvae in that replicate. We divided the number of larvae in each group by the total number in the replicate to determine the percentage of larvae that got stuck at the blade or passed through the particle plate for each group.

We determined the expected total number of larvae in each subsample by dividing the mass of the subsample by the average mass of a single larva from that batch. We determined the likelihood of instantaneous death for each subsample by multiplying the number of larvae cut in a particular body segment by the proportion of larvae that were immobile 1 s after being cut in that body segment during our individual mechanical separation trials; we then divided this number by the expected total number of larvae in the subsample. We determined the total likelihood of humane death using the proportion of larvae that got stuck at the blade vs made it through the plate and the likelihood of humane death for each of those groups.

Statistical analysis

Data were analysed in GraphPad Prism v 9.5.1 and R v 4.1.3 (R Core Team 2019; GraphPad Software for Windows 2023); normality and homoscedasticity were assessed prior to choosing a particular analysis method. A two-way ANOVA was used to assess the effects of larval body size and particle size plate hole diameter on loading and output rates in grams per second (g s^{-1}) and individuals per second. A mixed-effect model with Sidak’s multiple comparisons test was used to assess if paired larval output rates were slower than loading rates across all treatments; a linear regression was used to determine the relationship between loading and output rates. A one-way ANOVA was used to assess variance

in larval weight among treatment groups in the razor-cuts experiment. Alpha was set at $P < 0.05$.

A beta regression (Cribari-Neto & Zeileis 2010) was used to analyse the effects of trial and larval size on proportion of instant deaths. Model terms were also assessed with a type II ANOVA (Fox & Weisberg 2019). There was no significant interaction between trial and larval size when predicting proportion of humane deaths ($\chi^2_{2,1} < 0.01$; $P = 0.93$) nor was there a significant difference between trials ($\chi^2_{2,1} < 0.1$; $P = 0.31$), indicating there was no trial effect. However, we included trial as a random effect in all models regardless, to control for any unaccountable differences between trials.

Analysis of instant death percentages overall (stuck at the blade and passed through the plate, combined), instant death percentages at the blade only, and instant death percentages passed through the particle plate only, were conducted using generalised linear models with a beta family and logit-link to account for the dependent variable being percentage data (Brooks *et al.* 2017). Whenever percentage (dead instantly) was estimated to be less than 0%, which occurred sometimes in the 'stuck at blade' population due to variance in larval weight, a 0% instant death percentage was substituted. Trial number was fitted as a random intercept. Maximal models with interaction terms were fitted initially followed by step-wise removal of non-significant model terms as determined by log-likelihood tests. Pair-wise *post hoc* comparisons were conducted using the package 'emmeans' (Lenth 2023).

A generalised linear model with a beta family and logit-link were also used to analyse if changes in the likelihood of getting stuck at the blade were based on larval body size or particle size plate hole diameter. Trial was fitted as a random intercept and *post hoc* pair-wise comparisons were conducted using the package emmeans.

Data, videos, and all supplementary files are available at Barrett *et al.* (2023c).

Ethical justification

Research on insects is not currently subject to any legally mandated ethical review; therefore, no ethical approval was required to conduct this study. The authors made an effort to consider minimising the number of individuals used (e.g. by selecting only two body mass categories for the at-scale work and running pilot tests to determine the minimum sample size that could be run to attain accurate results of a full grinder). Individuals could not be anaesthetised/stunned prior to receiving cuts (as this would interfere with the behavioural assessment of death) or prior to the slaughter process (as larvae are not stunned on farms and larval activity during slaughter could have effects on survival that we should replicate). However, larvae were frozen following experimentation and sample collection to ensure death occurred rapidly. Notably, there is currently disagreement as to whether freezing provides a humane method of killing for insects, however current best practice guidelines do suggest this method can still be used until further data are acquired to resolve this dispute (Fischer *et al.* 2023). Despite our best efforts to consider and minimise welfare impacts on our research subjects we acknowledge a practice that we believe would improve our methods and further minimise harm to the animals in future studies that seek to replicate our design. Larvae could have been anaesthetised immediately following output from the slaughter machine (or immediately following the completion of the cut assay), preferably using isoflurane. This approach would prevent possible suffering during sample collection and weighing, which occurred while larvae were not

Table 1. Mean mass, and mass ranges, of larvae from each age/size group in g

Group	Mean (\pm SD) mass (g)	Mass range (g)
7 DOL	0.0063 (\pm 0.0029)	0.0013–0.0132
Medium	0.0956 (\pm 0.017)	0.0542–0.1480
Large	0.1573 (\pm 0.0232)	0.1032–0.2179
23–24 DOL	0.2346 (\pm 0.0307)	0.1565–0.2985

anaesthetised in our at-scale study, as well as any possible suffering during the freezing process.

Results

Body-size variation in the proportion of instantaneous death due to mechanical separation of the body segments

There was no statistically significant difference in the masses of larvae across treatments in any age/size group (One-way ANOVAs, 7 DOL: $F = 0.67$, $df = 12$; $P = 0.78$; medium: $F = 1.14$, $df = 12$; $P = 0.34$; large: $F = 0.91$, $df = 12$; $P = 0.54$; 23–24 DOL: $F = 0.79$, $df = 12$; $P = 0.66$); therefore, the masses of each group were calculated from all BSFL used in all treatments (Table 1). There was a degree of overlap in mass range in the medium and large, and large and 23–24 DOL, treatments (Figure S1; Supplementary File 3).

The proportion of BSFL that died instantly, or after 90 s, varied based on larval body size and treatment (e.g. body segment injury location; Figure 1, Tables S1, S2; Supplementary File 3). Smaller larvae were more likely to die across all treatments (Figure 1[b], [d]).

Across the four age/size categories, 160 BSFL were cut in body segments 9–12 where they could be expected to reach their open body cavity with their mouthparts. In observing their behaviours for 90 s, only one larva ever attempted to self-cannibalise (a 0.09-g 'medium' sized larvae), or 0.63% of the tested subjects. Other behavioural responses, exhibited by larvae across age/size categories and treatments, included: unprompted forward and backward locomotion; continuous lateral rolling; waving of the head from side-to-side; contracting the body segments; thrashing; rolling; circling of the head or posterior; as well as any of these movements prompted in response to a poke from the probe after a period of stillness (for examples of these behaviours, see Supplementary Videos 1–10 in Barrett *et al.* 2023c).

Loading and output rates

The loading rate of larvae in g s^{-1} to the machine did not vary according to body size or particle size plate hole diameter (two-way ANOVA; all $P > 0.05$; Figure S2; Supplementary File 3). Larvae were loaded at a rate of $39.4 (\pm 6.5) \text{ g s}^{-1}$ across all conditions. Large-sized larvae were loaded at a rate of $227.0 (\pm 30.9)$ estimated individuals per second, while medium-sized larvae were loaded at a rate of $369.7 (\pm 72.6)$ estimated individuals per second (Figure 2).

The rate of larval output in g s^{-1} from the machine also did not vary according to body size or particle size plate hole diameter (two-way ANOVA; all $P > 0.05$; Figure S2; Supplementary File 3). Larval output rates were $25.22 (\pm 4.41) \text{ g s}^{-1}$. Larval output rates were always slower than their loading rates (mixed-effect model: $X^2 = 11.16$, $df = 1$; $P = 0.0008$; Sidak's MCT: large: $t = 18.02$, $df = 17$; $P < 0.0001$; medium: $t = 9.59$, $df = 17$; $P < 0.0001$; Figure S3; Supplementary File 3). The large-sized larval output rate was $150.2 (\pm 20.0)$ estimated individuals per second while the medium-sized

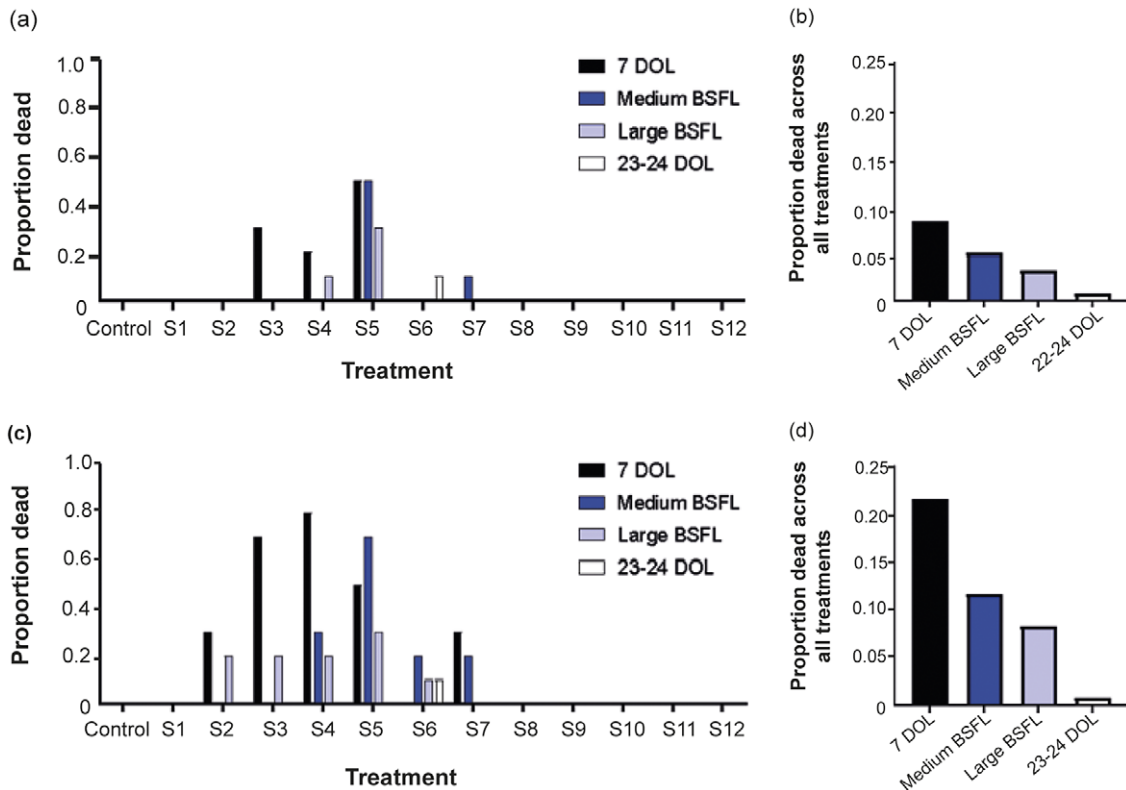


Figure 1. Proportion of larvae of different ages/sizes dead instantly, or by 90 s, due to cuts from a razor blade at different body segments. The proportion of larvae dead by body segment cut location, (a) instantly or (c) by 90 s; and the proportion of larvae dead across all treatments (cuts in S1–S12) (b) instantly or (d) by 90 s by age/size. DOL = day old larvae; BSFL = black soldier fly larvae.

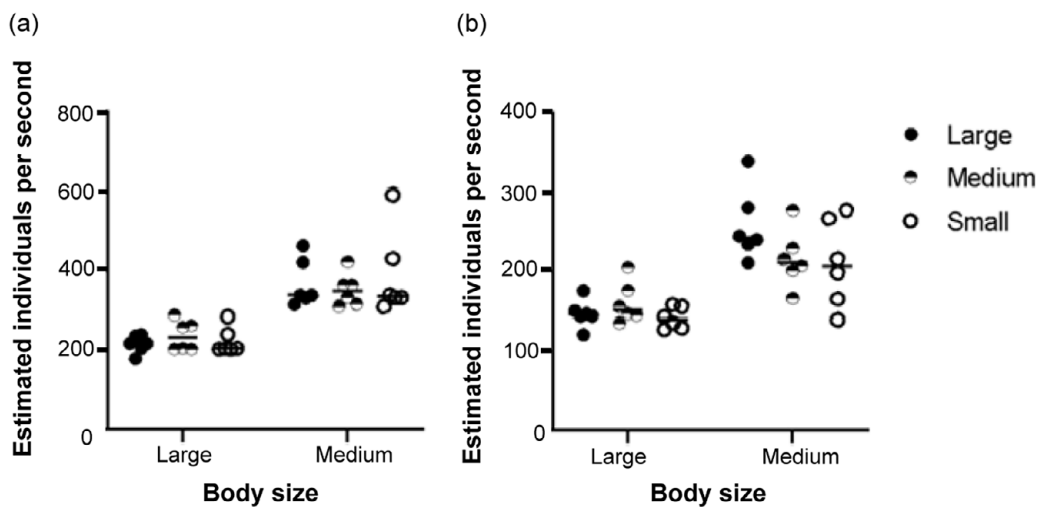


Figure 2. Estimated number of individual black soldier fly larvae (BSFL) processed per second using the grinding technique. Showing (a) estimated number of individual larvae of a large and medium body mass loaded per second into the hopper of the grinder and (b) estimated grinder output rate for large- and medium-sized larvae.

larval output rate was 228.6 (\pm 48.8) estimated individuals per second (Figure 2).

Effects of body size and particle size plate hole diameter on likelihood of instantaneous death

Medium-sized larvae from batches one and two weighed 0.1076 (\pm 0.0061) and 0.1055 (\pm 0.0043) g, respectively, while large-sized

larvae from these two batches weighed 0.1724 (\pm 0.0079) and 0.1749 (\pm 0.0066) g. Both were within the SD of the means of our medium- and large-sized populations used for the mechanical separation by body segment test.

A greater proportion of BSFL got stuck at the blade and did not make it through the particle size plate, if a smaller particle size plate hole diameter was used (Figure 3; $Z < -3.78$; $P < 0.001$), or if the larvae were smaller in body size (Figure 3; z ratio = 2.47; $P < 0.02$).

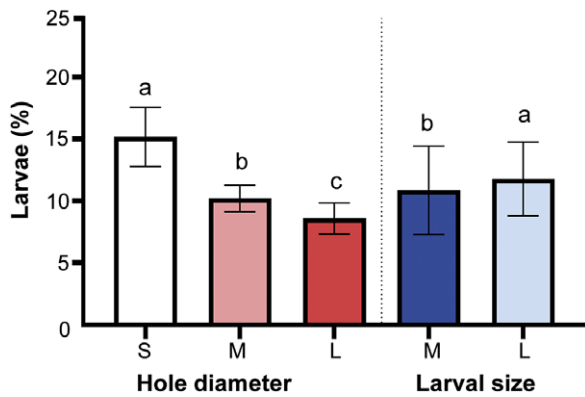


Figure 3. Percentage of black soldier fly larvae stuck at the blade relative to particle size plate hole diameter or larval body size, and likelihood of instantaneous death. A greater percentage of larvae got stuck at the blade when a smaller particle size plate hole diameter was used (Generalised linear model; S-M: $Z = -9.98$; $P < 0.0001$; S-L: $Z = -13.59$; $P < 0.0001$; M-L: $Z = -3.78$; $P = 0.0005$). Further, a greater percentage of large-sized larvae were stuck at the blade than medium-sized larvae (Generalised linear model; $Z = -2.47$; $P = 0.013$). Letters indicate statistically significant differences between hole diameters or larval sizes, with $P < 0.05$.

The (mean \pm SD) of all medium-sized larvae that got stuck at the blade when using the smallest plate hole diameter was 14.99 (\pm 3.21)% compared to 9.81 (\pm 1.13)% with the medium hole diameter, and 8.19 (\pm 1.51)% with the largest. For the large-sized larvae we saw 15.69 (\pm 1.49), 10.84 (\pm 0.81) and 9.19 (\pm 0.79)% stuck at the blade with the smallest, medium and largest plate hole diameter, respectively. The likelihood of instantaneous death for BSFL that got stuck at the blade did not vary based on particle size plate hole diameter or larval body size ($Z > -2.32$; $P > 0.05$). Across all larvae, only 2.65 (\pm 3.68)% showed instantaneous death when stuck at the blade (range: 0.00–13.12%).

A greater percentage of BSFL made it through the particle size plate, the larger the hole diameter ($Z < -12.38$; $P < 0.0001$). The likelihood of instantaneous death for larvae that made it past the particle size plate varied based on particle size plate hole diameter, but not larval body size (Figure 4; $Z = 1.26$; $P = 0.21$). In terms of experiencing an instantaneous death, we observed 99.27 (\pm 0.69)% with the smallest plate hole diameter and 84.97 (\pm 2.33) and 59.26 (\pm 3.10)% with the medium and largest, respectively.

The overall likelihood of experiencing an instantaneous death (when considering both the larvae that made it through the plate

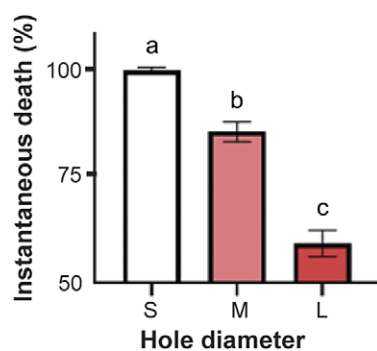


Figure 4. Instantaneous death for black soldier fly larvae relative to hole plate diameter. A greater percentage of larvae experience an instantaneous death when a smaller particle size plate hole diameter was used (Generalised linear model; S-M: $Z = -12.38$; $P < 0.0001$; S-L: $Z = -16.12$; $P < 0.0001$; M-L: $Z = -14.22$; $P < 0.0001$). Letters indicate statistically significant differences with $P < 0.0001$.

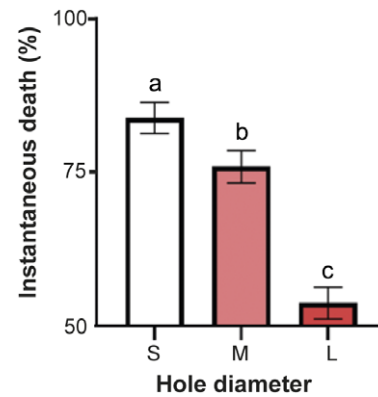


Figure 5. Instantaneous death for all black soldier fly larvae in a 500 g run (stuck at blade and past plate) relative to hole diameter. A greater percentage of larvae experience an instantaneous death when a smaller particle size plate hole diameter was used (Generalised linear model; S-M: $Z = -8.24$; $P < 0.0001$; S-L: $Z = -27.49$; $P < 0.0001$; M-L: $Z = -20.44$; $P < 0.0001$). Letters indicate statistically significant differences with $P < 0.0001$.

and those stuck at the blade) varied based on particle size plate hole diameter (Figure 5; $Z > -27.49$; $P < 0.0001$), but not larval body size (Figure 5; $X^2 = 0.01$; $P = 0.91$). Across all larvae in a run, 83.82 (\pm 2.57)% experienced an instantaneous death when using the smallest plate hole diameter with 76.03 (\pm 2.66) and 53.80 (\pm 2.62)% experiencing instantaneous death with the medium and largest hole diameter, respectively.

Finally, a total of 743 BSFL were found within the tube leading up to the blade at the end of runs, across all replicates; of these, 6.19% had received externally observable injuries (5.34 and 6.90% of medium- and large-sized larvae, respectively).

Discussion

We found that very few BSFL died instantly after receiving a cut to a single body segment. The segments in which cuts produced some likelihood of instantaneous death varied based on larval size, with smaller larvae (7 DOL) more likely to die instantly than larger larvae (23–24 DOL). This is likely a result of two factors: (1) nervous system location within the body cavity changing as the animals' grow non-isometrically in body and brain mass (Barrett *et al.* 2022); and (2) reduced oxygen availability due to proportionally increased haemolymph loss in smaller larvae. Larvae exhibited a variety of abnormal behaviours in response to mechanical separation of different body segments, including continuous rolling, thrashing, or backward locomotion. Despite widespread reports of insect self-cannibalism in response to injuries that rupture the body cavity (e.g. Eisemann *et al.* 1984), we found that only one larva (0.63% of subjects) engaged in this behaviour during our study (Supplementary Video 10 in Barrett *et al.* 2023c).

Particle size plate hole diameter, but not larval body size (across the range 106–175 mg in mass), affected the likelihood of instantaneous death when using a standard meat grinder design. The likelihood of instantaneous death was highest (84%) when the smallest particle size plate hole diameter was used (2.55 mm) and lowest (54%) when the largest particle size plate hole diameter was used (12 mm). However, a significant challenge in using this slaughter method to generate instantaneous death was the incomplete slaughter of a group of larvae that got stuck at the blade at the end of a run: only 3% of these larvae experienced an instantaneous death. If this

population was removed (e.g. all larvae made it through the particle size plate), instantaneous death was more likely across all plate sizes: 99% for the smallest plate and 59% for the largest.

From these data, we can make the following recommendations to producers to ensure a 99% chance of instantaneous death for their larvae when using grinding for slaughter:

- Validate mortality rates with each machine, using the parameters defined here;
- For grinding machines that use a particle size plate, use hole diameters 2.55 mm or smaller for BSFL populations that have a minimum average body mass of 106 mg;
- To prevent BSFL from being incompletely slaughtered (e.g. stuck at the blade), run a filler (substrate, dead BSFL, vegetables, etc) through the machine at the end of the run to ensure all BSFL are completely processed; and
- When using machines with other designs, or slaughtering BSFL smaller than 106 mg or larger than 175 mg in average mass, producers should test their specific SOP (see methodology and materials in Supplementary Files 1 and 2) to determine the likelihood of instantaneous death.

An important limitation of our results is that we were unable to assess any injuries that occurred in the machine prior to reaching the blade/plate, which could have contributed to pain/suffering prior to the time of death. We found that larvae spend 3.78 s in the machine (the time from pouring to output), meaning there are several seconds during which time larvae may incur an injury, prior to death at the blade/plate. Indeed, 6.19% of larvae found in the tube, anterior to the blade, at the end of runs were injured; however, given the unusual low-density loading of the machine at the very end of a run, it is unclear if these larvae are representative of the experiences of all larvae.

Grinding may also serve as a method for humane depopulation, should producers need to cull livestock in response to an emergency (e.g. disease outbreak). In cases of depopulation, insects may not be separated from their feeding substrate, while at times of slaughter (and thus in our study) larvae have been separated from their feed. Our results may not accurately reflect instances of depopulation where substrate would still be mixed in with larvae, or when larvae are smaller than 106 mg in mass (as larvae may need to be depopulated at very early instars). Until empirical data on these body sizes, and with substrate mixed in, are tested, we recommend producers monitor their own grinding-depopulation SOPs via modification of the previously mentioned methodology and materials in Supplementary Files 1 and 2.

Overall, our results suggest that grinding can serve as an instantaneous method of slaughter for 106–175 mg BSFL, but that SOP strongly affects the probability of humane death. Small particle size plate hole diameters, fillers at the ends of runs to reduce the probability of incomplete slaughter, and testing of each novel machine design or larval body size can ensure grinding is used humanely for the slaughter of BSFL. Furthermore, determining if the method is viable in terms of economics and practicality should also be considered. Finally, while grinding may serve as an instantaneous method of slaughter, it may still lead to public scrutiny of insect slaughter practices due to negative public perception of methods employed; further research into the social acceptability of this practice for insect farming would be valuable.

Animal welfare implications

Currently, billions of farmed BSFL are slaughtered each year with no established SOPs to ensure instantaneous death. This study

provides the first empirically supported guidance for humane processing of BSFL; implementation of these practices by producers would help reduce the potential risk of unnecessary suffering for billions of animals.

Supplementary material. The supplementary material for this article can be found at <http://doi.org/10.1017/awf.2024.10>.

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