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ORIGINAL ARTICLE

Black soldier fly (Diptera: Stratiomyidae) larval heat generation and management

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Abstract Mass production of black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), larvae results in massive heat generation, which impacts facility management, waste conversion, and larval production. We tested daily substrate temperatures with different population densities (i.e., 0, 500, 1000, 5000, and 10 000 larvae/pan), different population sizes (i.e., 166, 1000, and 10 000 larvae at a fixed feed ratio) and air temperatures (i.e., 20 and 30 °C) on various production parameters. Impacts of shifting larvae from 30 to 20 °C on either day 9 or 11 were also determined. Larval activity increased substrate temperatures significantly (i.e., at least 10 °C above air temperatures). Low air temperature favored growth with the higher population sizes while high temperature favored growth with low population sizes. The greatest average individual larval weights (e.g., 0.126 and 0.124 g) and feed conversion ratios (e.g., 1.92 and 2.08 g/g) were recorded for either 10 000 larvae reared at 20 °C or 100 larvae reared at 30 °C. Shifting temperatures from high (30 °C) to low (20 °C) in between (∼10-d-old larvae) impacted larval production weights (16% increases) and feed conversion ratios (increased 14%). Facilities should consider the impact of larval density, population size, and air temperature during black soldier fly mass production as these factors impact overall larval production.

Key words Allee effect; density; *Hermetia illucens*; industry; substrate heat; thermal ecology

Introduction

Black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), larvae are used to recycle organic wastes while also producing insect biomass for use as animal feed globally (Raman *et al.*, 2022). While there is an industrial scale (e.g., at least 10 000 larvae/pan) of production commonly used much information available for developing appropriate procedures for such processes are based on benchtop-scale experiments (Harnden & Tomberlin, 2016; Holmes *et al.*, 2016; Chia *et al.*, 2018). Scale discrepancy leads to challenges when estimating the efficiency and value of such systems. As an example, larval production at an industry scale differed 0.6% from the benchtop, which was calculated to have a financial miscalculation of $250 000 USD annually based on a farm with a daily feedstuff conversion capacity of 20 tons (Yang & Tomberlin, 2020). One potential explanation for the difference in larval performance between industry and benchtop production could be heat generated by the larvae varying across systems.

During black soldier fly larval-mass rearing, the larvae themselves generate excess heat through their movements and metabolism (Shishkov, 2020). In some

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instances, the excess heat exceeds their upper thermal threshold (e.g., 47 °C), resulting in reduced larval growth and in some cases partial or complete larval mortality if they are not removed from their containers (Ewusie *et al.*, 2019). Thermal images have documented that certain positions within black soldier larval masses reach 45 °C (Müller *et al.*, 2017). However, it is not clear whether these elevated temperatures are the result of larval activity, population density, microbial activity, or a combination of these factors.

The concept of maggot mass temperature is not new. Necrophagous dipteran larvae usually feed in aggregations leading to much higher larval mass temperatures than air temperatures, which is also known as the maggot-mass effect (Matuszewski & Mądra-Bielewicz, 2021). Blow flies (Diptera: Calliphoridae), such as *Lucilia sericata* Meigen, colonize carrion and can increase substrate temperatures by ∼4 °C (Matuszewski & Mądra-Bielewicz, 2021). In a similar study, blow fly larval aggregations generated heat resulting in a ∼20 °C increase above ambient temperature (Charabidze *et al.*, 2011). Interestingly, such responses do not require large numbers of larvae; populations as low as 100 individuals have been able to generate heat (∼12 °C above air temperature at 24 °C) (Kotzé *et al.*, 2016). In fact, 2000 black soldier fly larvae can increase diet (chicken feed mixed with 50% water by volume) temperature by 8 °C within 78 min after being introduced, while their metabolism increased from 0.4 mW/larva to 0.6 mW/larva (Shishkov, 2020).

While some increases in temperature are deleterious (e.g., reduced growth and death; see citations above), some instances can be favorable. Aggregation is a recognized strategy used by many organisms to increase fitness. Through aggregation, benefits range from faster developmental rates and greater foraging efficiencies to better survival against predators or pathogens (Ermolaev *et al.*, 2020). For example, larvae of the blow fly, *Calliphora vicina* Robineau-Desvoidy, developed ∼30 h faster when reared at higher density (e.g., 1000 vs. 50) (Kökdener *et al.*, 2019). Such phenomena are examples of an Allee effect, where the fitness of an individual correlates to larval density (Merritt & De Jong, 2015; Komo *et al.*, 2021). The heat generated during aggregation is one of the benefits from the Allee effect. Other benefits include increased production of digestive enzymes and antibiotics (Descalzi, 2019). While larvae can benefit from heat, if temperatures increase too much, negative outcomes are encountered (Rivers *et al.*, 2011), for example higher mortality (Chia *et al.*, 2018). Thus, from an industry perspective with insects that are mass produced, managing temperature is a tightrope that can be either beneficial or detrimental biologically and consequently, financially.

Larva Larval death from overheating is observed during the mass production of black soldier fly larvae when they are in confined environments (e.g., rearing pans) that do not allow for behavioral thermoregulation and do not draw the excess heat away fast enough to keep the larvae from overheating (Shishkov, 2020). Therefore, determining the parameters (e.g., larval density, temperature over time) driving heat production can potentially lead to methods allowing industry to capture the positive outcomes (e.g., high survival, quick development, high conversion rates) while avoiding the potential pitfalls (e.g., overheating, low survival, low conversion rates).

This study aimed to determine: (1) how larval population densities impact substrate temperatures, which are highly correlated with larval body temperature; (2) how larval population sizes and air temperatures impact substrate temperatures and subsequent larval performance; and (3) whether the larval production can benefit from shifting air temperatures at specific stage in the larval growth process. We measured substrate temperature and larval growth performance at different larval densities (i.e., same container size, different larval quantity), population size (i.e., different container size, same larval quantity per unit volume), and air temperatures to simulate different larval rearing situations. We hypothesized that population density, population size, and air temperature can impact substrate temperatures resulting in larval performances variation.

Materials and methods

Colony maintenance

Every generation was started from from a Bullet® (Tomberlin *et al.*, 2021) consisting of ∼10 000 7-d-old larvae. After feeding them with 8 kg Gainesville diet (containing 50% wheat bran, 30% alfalfa meal, 20% corn meal, and moisturized to 70% water content; Hogsette, 1992), mature larvae were sifted for pupation. After pupae emerged, adults were transferred to a greenhouse in F.L.I.E.S Facility (Texas A&M University, College Station, Texas, USA) for reproduction. Detailed colony management procedures were described by Li *et al.* (2022) and Addeo *et al.* (2022). Black soldier flies used in this study were raised from eggs collected from the colony maintained in the greenhouse (described above).

Heat generation measurement

This experiment was done in a laboratory walk-in environmental chamber set to 27.0 ± 0.5 °C, 60% ± 5.1% relative humidity (RH), 14 : 10 light to dark cycle

(L : D). Eggs were hatched in the walk-in chamber (described above) and newly hatched larvae were weighed with an electronic balance (Ohaus® Adventurer™ Pro, model AV264C, Parsippany, USA) as a method of counting. To test the larval density effect, the number of larvae for every 8 kg of diet were increased from 0 (control) to 10 000 (i.e., regular lab rearing protocol, Addeo *et al.*, 2022). Different larval quantities (i.e., 0, 100, 500, 1000, 5000, and 10 000 larvae/pan) were separated according to weight, and the accordant 1-d-old larvae were transferred to a Kerr® wide-mouth 946 mL Mason Jar (Hearthmark LLC, Daleville, IN, USA) with 100 g wet Gainesville diet for a 7-d small-container larval incubation in a walk-in chamber. On day 7, larvae with their residue were placed with 8 kg wet Gainesville diet in a larger plastic rectangle pan (59.7 cm × 42.9 cm × 14.9 cm, Sterilite®, Townsend, MA, USA). An additional (0.7 kg) dry Gainesville diet was applied as a barrier along the edge of container to prevent larvae escaping their container. Substrate temperatures were measured daily using a four-channel thermometer with type K thermocouples (Ametek Arizona Instrument LLC, Chandler, AZ, USA). Throughout the experiment, temperatures at half depth in the substrate were measured at five locations (i.e., four near the container wall/corner and one in the middle; please see the red crosses in the large pan in Fig. S1) once daily at 10 am. Since day 8, thermal images (Model T650sc, Teledyne FLIR LLC) were taken from directly over each container. Through the thermal image, the surface hottest location was determined for each container daily and the hot spot temperature was measured at half depth inside the substrate. Maximum substrate temperature, the average substrate temperature (i.e., average data from the four corners and the center), and the substrate temperature variation (i.e., the difference between the greatest and lowest temperature recorded daily in each pan) were recorded. The harvest day for each pan was determined by observing two consecutive days of temperature declines. Three trials were done with eggs hatched from different generations in summer (n total = 6 population density × 3 replicates × 3 trials = 54).

Heat management experiment

Six treatments (i.e., 20L, 30L, 20M, 30M, 20S, and 30S) were tested with a combination of two air temperatures (i.e., 20 and 30 °C) and three population size [e.g., 10 000, 1000, and 166 larvae for large (L), medium (M), and small (S), respectively; Fig. S1]. Furthermore, the three population sizes were placed in three different sized containers: Sterilite® pan, a cylinder plastic container (~1893 mL, Airlite® 6451, Omaha, NE, USA), and a small cup (~473 mL, Great Value® from Walmart, AR, USA) for L, M, and S population size. The population size was determined to ensure the three sizes of container had the same larval density. Gainesville diet (70% water content) was added to each container for substrate with a proportion of 8 g per 10 larvae. Containers were chosen specifically to result in similar depth (~6 cm) of diets across treatments within the three different types of containers. Black soldier fly larvae (7-d-old) were added to each container and placed in growth chambers (Percival Scientific Inc. Perry, IA, USA, model DR36VL, with RH set at 60% and L : D = 14 : 10) set at either 20 or 30 °C. Average daily larval weights were measured by randomly selecting 10 larvae from each container and weighing them individually on an electronic balance (mentioned above). The average larval weight was noted daily until two consecutive days of weight loss were recorded. At that time, larvae were all removed from the container and survival was determined gravimetrically. As suggested by Waldbauer (1968), the feed conversion ratio (i.e., FCR = feed added in dry weight/larvae harvested in fresh weight), digested feed conversion efficiency [i.e., ECD = (feed added in dry weight − residue in dry weigh)/larvae harvested in dry weight], and approximate digestibility [i.e., AD = (feed added in dry weight − residue in dry weight)/feed added in dry weight] were determined. Substrate temperatures were measured once daily at 10 am using a four-channel thermometer with type K thermocouples. For medium and small containers, only the center temperatures were measured, while for the large containers, temperatures at the center as well as at four corners (5 cm away from the corner) were measured due to the large thermal heterogeneity within a container. The maximum temperature and the average temperature were recorded. Meanwhile thermal pictures (Ulefone, Armor 9 Android smartphone, Shenzhen, China) were taken as a reference of the surface temperature (n total = 2 temperatures × 3 population sizes × 3 replicates × 2 trials = 36).

Temperature shifting experiment

Similar to the treatment set up above, the same three pan sizes (S, M, and L described in Heat management experiment) were inoculated with 8 kg Gainesville diet and 10 000 7-d-old black soldier fly larvae per pan. Four treatments differed only in the air temperature were tested: (1) larvae reared at 30 °C for the entire test referred as control; (2) larvae reared at 30 °C for 2 d and shifted to 20 °C until harvest day was determined; (3) larvae reared at 30 °C for 4 d and shifted to 20 °C until harvest day

was reached; (4) larvae reared at 20 °C for the entire test. With similar procedure in the heat management experiment, daily average larval weight, total larval weight at harvest, duration until harvest day occurred, and FCR were recorded (n total = 4 treatments × 2 replicates × 2 trials = 16).

Statistics

For the heat generation experiment, first, interaction effects between trial and population density were checked with mixed linear models (i.e., ∼larval age × population density + trial × population density) with the replicate ID as the random effect due to using repeated measurements. After interaction effects between trial and population density were excluded, the impacts of larval age and population density on the maximum substrate temperature, average substrate temperature, or substrate temperature variation were compared using mixed linear models (i.e., ∼larval age × population density + trial) with the replicate ID as the random effect due to using repeated measurements. Function "lme" in package "nlme" was used (Pinheiro *et al.*, 2017). Restricted maximum likelihood (REML) was used as the model fitting method to avoid biased estimations. Model assumptions were checked with diagnostic plots. Pairwise comparisons among population density at each larval age were done with "emmeans" function in "emmeans" package (Lenth, 2022). For the heat management experiment, the Scheirer–Ray–Hare test was applied to detect the main effects as well as any interaction of the temperature and population size. Kruskal–Wallis test followed with Dunn's test were used for pairwise comparison. For the temperature shifting experiment, Wilcoxon rank-sum test was applied for treatment comparison against the control. All the statistical analyses were done in R version 4.0.5 (R Core Team, 2015) with the significance level set at $P < 0.05$.

Results

Heat generation

Significant trial effects were detected for all three dependent variables (e.g., maximum substrate temperature, average substrate temperature, and substrate temperature variation). No significant ($P > 0.05$) interaction effects between trial and other independent variables were detected. The interaction between population density and larval age were significant for all the three dependent variables, which are maximum substrate temperature

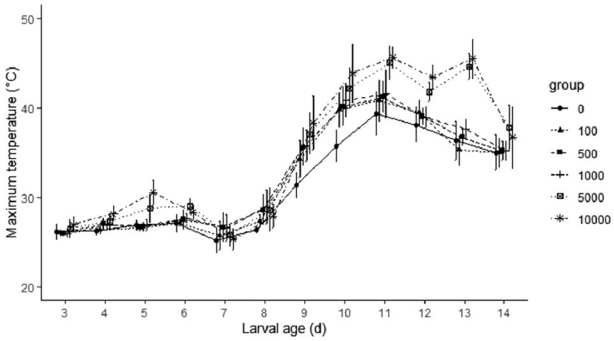

Fig. 1 The daily maximum temperature (mean ± CI) in substrate with different black soldier fly larval density (0−10 000 larvae/pan with 8 kg diet) placed in a walk-in chamber under 27.0 ± 0.5 °C, 60% ± 5.1% RH, 14 : 10 L : D.

($F_{55,\,510} = 5.11$, $P < 0.001$), average substrate temperature ($F_{55,\,510} = 5.35$, $P < 0.001$), and substrate temperature variation ($F_{55,\,510} = 2.55$, $P < 0.001$). In general, the greater the population density, the greater the maximum substrate temperature (Fig. 1) and average substrate temperature (Fig. S2) were. The maximum substrate temperature increased with larval age (e.g., started from ∼26 °C near room temperature), peaked around day 11 (e.g., between 39 and 46 °C) (i.e., which is equivalent to 11-d-old larvae and the fourth day after shifting from small cups to big pans, with dimensions described above), and then started to decrease (e.g., between 35 to 38 °C at the harvest day). Population density effects were greater from day 9 to day 13, within which the greatest maximum substrate temperature (45.6 °C) and average substrate temperature (43.2 °C) was recorded in the group with 10 000 larvae/pan at day 11. Notably, the group with pure substrate (i.e., no larvae) also had high substrate temperatures (39.3 °C at maximum and 37.1 °C on average at day 11). Substrate temperature variations varied from 0.9 °C (population density of 0 larvae/pan at day 8) to 7.9 °C (population density of 5000 larvae/pan at day 13) in pans (Fig. S3).

Heat management

In general, the temperature effects are mostly population size dependent, within which the most promising results (e.g., regarding larval weight and conversion efficiency) were determined in the large population size at 20 °C. For growth performance traits, rearing durations were shorter ($H = 27.49$, df = 1, $P < 0.001$) at 30 °C (7.0−7.8 d) then those at 20 °C (9.2−13.7 d) in general. At 20 °C, large population size can reduce duration by 4 d at least compared to smaller population size at the same

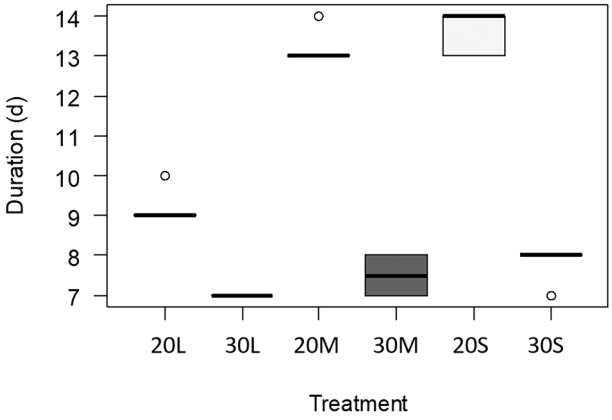

Fig. 2 Rearing duration with different black soldier fly larval population size (L, M, and S for 10 000, 1000, and 166 larvae) and air temperature (20 and 30 °C). Shades in box plots represent the first to third quartile. Scheirer–Ray–Hare test was applied to detect the main effects as well as any interaction of the temperature and population size. $H = 27.49$, df $= 1$, $P < 0.001$ between air temperatures. $H = 4.75$, df $= 2$, $P = 0.093$ among population sizes.

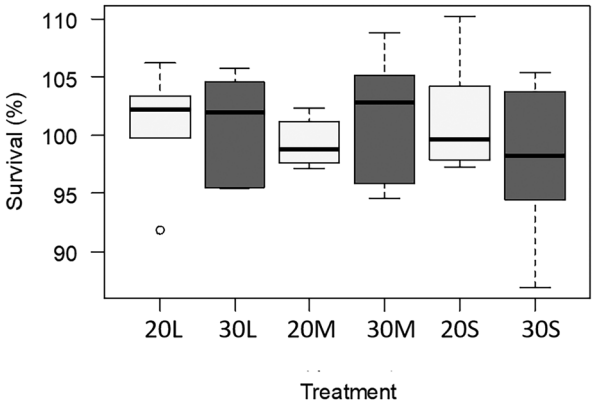

Fig. 3 Larval survival rates with different black soldier fly larval population size (L, M, and S for 10 000, 1000, and 166 larvae) and air temperature (20 and 30 °C). Shades in box plots represent the first to third quartile. Scheirer–Ray–Hare test was applied to detect the main effects as well as any interaction of the temperature and population size. $H = 0$, df $= 1$, $P = 1.000$ between air temperatures. $H = 0.54$, df $= 2$, $P = 0.763$ between population sizes.

temperature (Fig. 2). No significant differences were found on survival rates among treatments ($\chi^2 = 2.16$, df $= 5$, $P = 0.827$) (Fig. 3). There was an interaction effect ($H = 24.01$, df $= 2$, $P < 0.001$) between temperature and population size on average larval weights, which decreased with decreasing population size at 20 °C (0.097−0.126 g per larva) and increased with decreasing

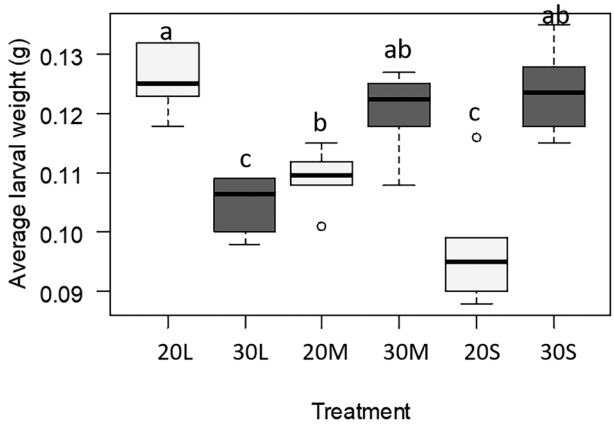

Fig. 4 Average larval weight with different black soldier fly larval population size (L, M, and S for 10 000, 1000, and 166 larvae) and air temperature (20 and 30 °C). Shades in box plots represent the first to third quartile. Scheirer–Ray–Hare test was applied to detect the main effects as well as any interaction of the temperature and population size. $H = 24.01$, df $= 2$, $P < 0.001$ for the interaction between air temperatures and population sizes. Kruskal–Wallis test followed with Dunn's test were used for pairwise comparison. Different letters indicate statistical differences between groups.

population size at 30 °C (0.105−0.124 g per larva). The largest larvae (0.126 g) were recorded at 20 °C with the large population size, while the smallest larvae (0.097 g) were recorded at 20 °C with the small population size (Fig. 4). For nutritional indices, interaction effects were detected in FCR ($H = 23.80$, df $= 2$, $P < 0.001$) and ECD ($H = 12.15$, df $= 2$, $P = 0.002$), but not in AD ($H = 0.82$, df $= 2$, $P = 0.662$). FCR increased with decreasing population size at 20 °C (1.92−2.79 g/g), but an opposite trend was observed at 30 °C (2.08−2.30 g/g). Treatment 20L had a lowest FCR (1.92 g/g) (Fig. 5). ECD decreased with decreasing population size at 20 °C (0.18−0.25 g/g), but no differences of ECD among population size were found at 30 °C (0.22−0.23 g/g) (Fig. 6). AD (0.55−0.61 g/g) was impacted by population size ($H = 17.65$, df $= 2$, $P < 0.001$) significantly but not by temperature ($H = 2.29$, df $= 1$, $P = 0.13$) (Fig. 7). For temperature indices, no interaction effects were detected. The maximum temperatures (24.33−42.37 °C) decreased with decreasing population size ($H = 25.31$, df $= 2$, $P < 0.001$) and temperature ($H = 5.12$, df $= 1$, $P = 0.024$). Small population size had maximum substrate temperatures ~4 °C greater than the associated air temperatures, medium population size had maximum substrate temperatures ~8.5 °C greater than the associated air temperatures, but notably, large population size at both 20 and 30 °C achieved the greatest maximum temperature around 43 °C (e.g., 23 and 13 °C above

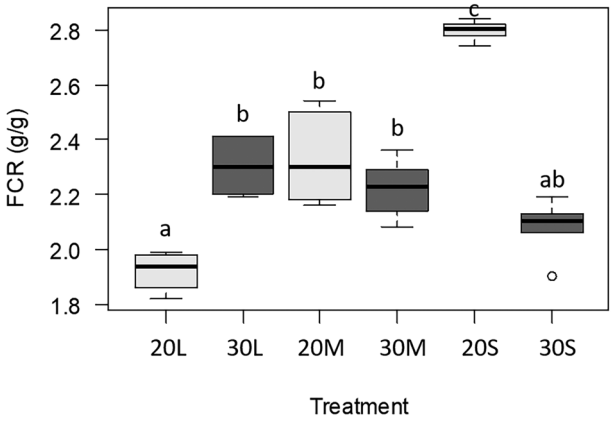

Fig. 5 Feed conversion ratio (FCR = feed dry weight/larval fresh weight gain) with different black soldier fly larval population size (L, M, and S for 10 000, 1000, and 166 larvae) and air temperature (20 and 30 °C). Shades in box plots represent the first to third quartile. Scheirer–Ray–Hare test was applied to detect the main effects as well as any interaction of the temperature and population size. $H = 23.80$, df = 2, $P < 0.001$ for the interaction between air temperatures and population sizes. Kruskal–Wallis test followed with Dunn's test were used for pairwise comparison. Different letters indicate statistical differences between groups.

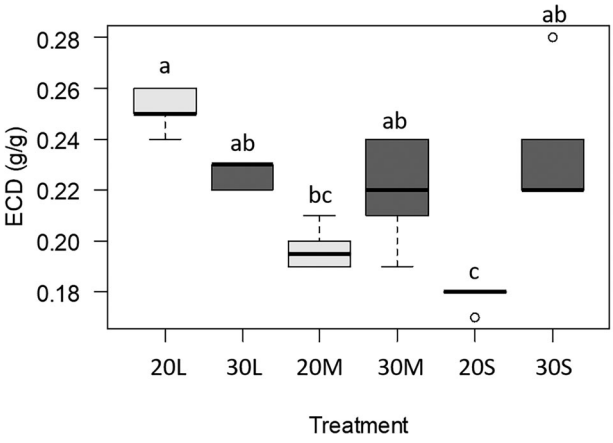

Fig. 6 Digested feed conversion efficiency [ECD = larval dry weight gain/(feed dry weight – frass dry weight)] with different black soldier fly larval population size (L, M, and S for 10 000, 1000, and 166 larvae) and air temperature (20 and 30 °C). Shades in box plots represent the first to third quartile. Scheirer–Ray–Hare test was applied to detect the main effects as well as any interaction of the temperature and population size. $H = 12.15$, df = 2, $P = 0.002$ for the interaction between air temperatures and population sizes. Kruskal–Wallis test followed with Dunn's test were used for pairwise comparison. Different letters indicate statistical differences between groups.

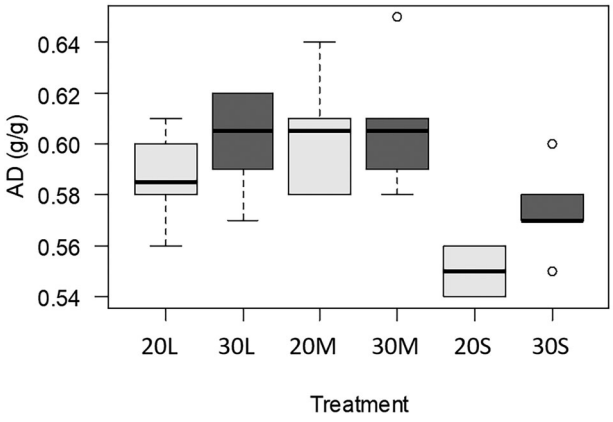

Fig. 7 Approximate digestibility [AD = (feed dry weight – frass dry weight)/feed dry weight] with different black soldier fly larval population size (L, M, and S for 10 000, 1000, and 166 larvae) and air temperature (20 and 30 °C). Shades in box plots represent the first to third quartile. Scheirer–Ray–Hare test was applied to detect the main effects as well as any interaction of the temperature and population size. $H = 2.29$, df = 1, $P > 0.05$ between air temperatures. $H = 17.65$, df = 2, $P < 0.001$ among population sizes.

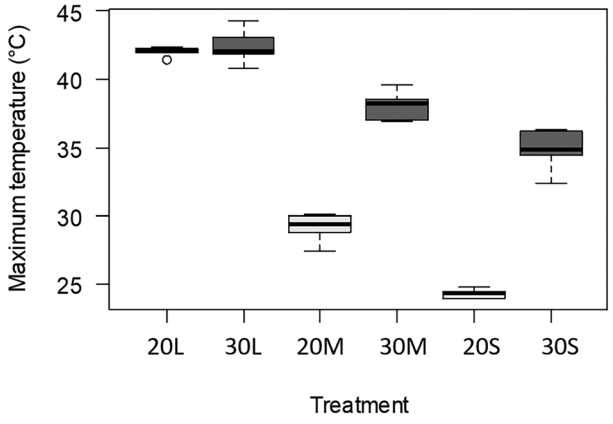

Fig. 8 Maximum substrate temperature with different black soldier fly larval population size (L, M, and S for 10 000, 1000, and 166 larvae) and air temperature (20 and 30 °C). Shades in box plots represent the first to third quartile. Scheirer–Ray–Hare test was applied to detect the main effects as well as any interaction of the temperature and population size. $H = 5.12$, df = 1, $P = 0.024$ between air temperatures. $H = 25.31$, df = 2, $P < 0.001$ among population sizes.

the associated air temperatures, respectively) without significant differences (Fig. 8). Similarly, the substrate average temperatures (22.22−34.13 °C) decreased with decreasing population size ($H = 7.57$, df = 2, $P < 0.02$) and air temperature ($H = 26.27$, df = 1, $P < 0.001$),

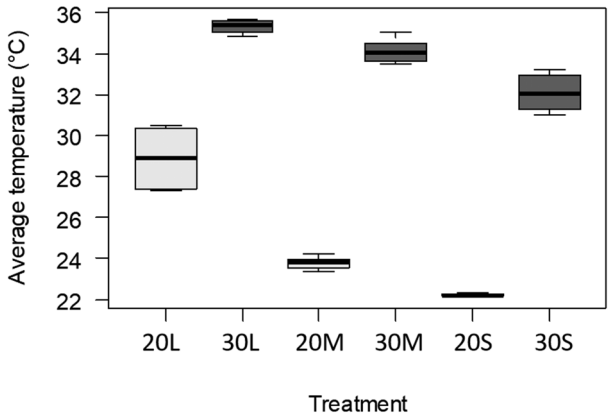

Fig. 9 Average substrate temperature with different black soldier fly larval population size (L, M, and S for 10 000, 1000, and 166 larvae) and air temperature (20 and 30 °C). Shades in box plots represent the first to third quartile. Scheirer–Ray–Hare test was applied to detect the main effects as well as any interaction of the temperature and population size. $H = 26.27$, df = 1, $P < 0.001$ between air temperatures. $H = 7.57$, df = 2, $P = 0.023$ among population sizes.

but with the temperature effect greater than the size effect, which is opposite to the maximum temperature data (Fig. 9). Larvae in large population size treatment aggregated at 20 °C causing a large thermal gradient in substrate, while larvae were more evenly distributed at 30 °C (surface temperature differences at day 6 shows in Fig. S5).

Temperature shifting

Temperature set at 30 °C (i.e., control) all through the entire test resulted in a total larval weight of 1.15 ± 0.04 kg with a FCR of 2.09 ± 0.07 g/g and a duration of 6.25 ± 0.48 d (mean ± SEM). Larvae reared at 20 °C had greater ($W = 16$, df = 1, $P = 0.014$) total weight (1.43 ± 0.09 kg), lower ($W = 16$, df = 1, $P = 0.014$) FCR (1.70 ± 0.10 g/g), and longer ($W = 15$, df = 1, $P = 0.024$) duration (7.75 ± 0.25 d) compared to larvae reared at 30 °C. Larvae shifted to 20 °C at day 9 had greater ($W = 16$, df = 1, $P = 0.014$) total weight (1.34 ± 0.03 kg), lower ($W = 16$, df = 1, $P = 0.014$) FCR (1.79 ± 0.04 g/g), and slightly longer but not significantly different ($W = 3$, df = 1, $P = 0.068$) duration (7.25 ± 0.25 d) compared to larvae reared at 30 °C. Larvae shifted to 20 °C at day 11 had greater ($W = 16$, df = 1, $P = 0.014$) total weight (1.34 ± 0.02 kg), lower ($W = 16$, df = 1, $P = 0.014$) FCR (1.80 ± 0.03 g/g), and similar ($W = 6$, df = 1, $P = 0.3245$) duration (6.75 ± 0.48 d) compared to larvae reared at 30 °C. (Figs. 10–12)

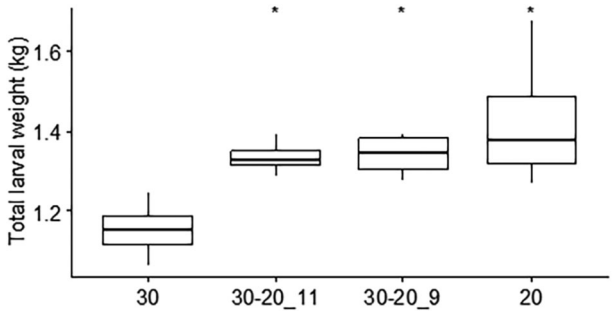

Fig. 10 Total black soldier fly larval weight for treatments at 30 °C (i.e., 30), 30 °C shifted to 20 °C at day 11 (i.e., 30-20_11), 30 °C shifted to 20 °C at day 9 (i.e., 30-20_9), and 20 °C (i.e., 20). Wilcoxon rank-sum test was applied for treatment comparison against the control (i.e., 30) with the significance level set at $P < 0.05$.

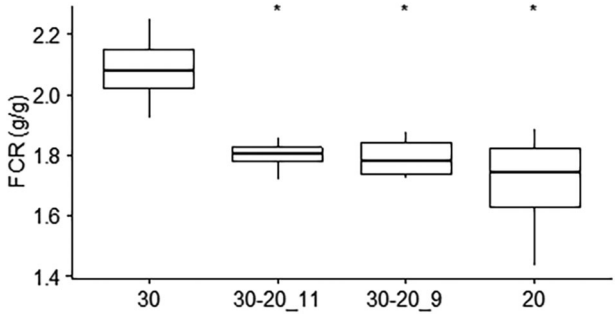

Fig. 11 Feed conversion ratio (FCR = feed dry weight/larval fresh weight gain) of black soldier fly larvae for treatments at constant 30 °C (i.e., 30), 30 °C shifted to 20 °C at day 11 (i.e., 30-20_11), 30 °C shifted to 20 °C at day 9 (i.e., 30-20_9), and constant 20 °C (i.e., 20). Wilcoxon rank-sum test was applied for treatment comparison against the control (i.e., 30) with the significance level set at $P < 0.05$.

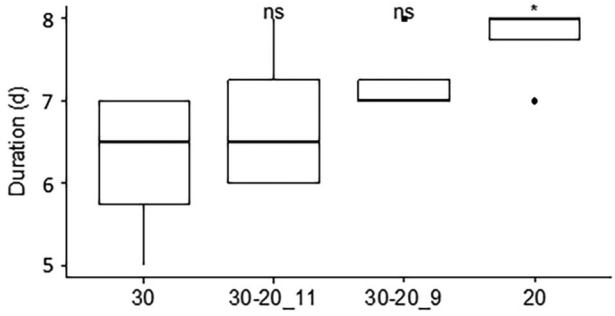

Fig. 12 Duration of black soldier fly larvae for treatments at 30 °C (i.e., 30), 30 °C shifted to 20 °C at day 11 (i.e., 30-20_11), 30 °C shifted to 20 °C at day 9 (i.e., 30-20_9), and 20 °C (i.e., 20). Wilcoxon rank-sum test was applied for treatment comparison against the control (i.e., 30) with the significance level set at $P < 0.05$. ns, not significant.

Discussion

This study demonstrated temperature management throughout the larval growth cycle is crucial for optimizing black soldier fly larval production. Heat generation data showed a clear pattern (e.g., bell-shape curves from day 8 to day 14) of substrate temperature and larval growth. Increased amounts of heat were generated as larvae aged, but then began to decrease when they started losing weight (i.e., stopped feeding). Such a universal change of substrate temperature partially reveals the metabolic change along larval ontogeny (McEachern, 2018) as well as the feed digestion activity. The mass specific metabolic rate of black soldier fly larvae has been determined to decrease from ∼12 to ∼2 μW/mg during larval development from the third to the last instar, while at the mean time the larval weight increases from ∼1 to 90 mg (Gligorescu *et al.*, 2019). Therefore, metabolic change per larva (e.g., the product of the body mass and the specific metabolic rate) followed a bell-shape curve as well (estimated by the Gligorescu *et al.*, 2019). Similarly, another black soldier fly study found that when larvae were older and larger more total heat from larvae was measured. However, at the same time less heat was produced per gram of body weight (McEachern, 2018). Therefore, the heat generated from larvae is at least larval age and larval weight dependent.

Positive correlations between larval density and substrate temperature were found. At room temperature (27 °C), the substrate maximum temperature (Fig. 2) and average temperature (Fig. S2) increased with increasing larval density (e.g., from 0 to 10 000 larvae/pan) with ∼8 °C difference between control and highest density (Fig. S4). The amount of heat generated is usually a result from multiple factors, such as larval age, larval weight, substrate volume, and location (Descalzi, 2019; Olea *et al.*, 2019; Chappell *et al.*, 2022). For example, large carcasses (e.g., 15 kg) that are decomposing and colonized by flies usually contain larger larval numbers, which can lead to greater internal temperatures due to metabolic activity, compared to small carcasses (e.g., 8 kg) with fewer larvae (Olea *et al.*, 2019). Interestingly, the control (i.e., no larvae) still had temperatures up to ∼10 °C above the air temperature (Fig. 1), which could be due to microbial activity (Rastogi *et al.*, 2020).

Substrate temperature variation within small cups (i.e., day 3 to day 7) was more consistent and minimal (e.g., <2 °C) compared to larger pans (i.e., day 8 to day 14) (5−10 °C). However, shifts in temperature across densities were relatively consistent (Fig. S3) indicating container size or the substrate surface to volume ratio is crucial in determining the substrate thermal heterogeneity at a certain air temperature regardless the larval density. A possible positive aspect of temperature variation within a pan is that it allows larvae to behaviorally thermoregulate. A potential negative aspect is that larvae will likely experience unsuitable temperatures when moving around the pan.

The heat management data indicate larval growth was density and air temperature dependent (Figs. 2−9). The greatest average larval weight (Fig. 4), lowest FCR (Fig. 5), and greatest ECD (Fig. 6) recorded for the treatment 20L (e.g., large population size at 20 °C) indicate larvae can effectively regulate their body temperature through locomotion. Though we did not record locomotion of singular larvae, we hypothesize, through locomotion, larvae in treatment 20L grew relatively fast with greater digestibility at higher temperatures while maintaining a relatively low metabolic rate at lower temperatures resulting in the best overall performance (Angilletta *et al.*, 2002; Heaton *et al.*, 2018).

Large (e.g., ∼10 000 larvae) larval population size can increase substrate temperatures (i.e., maggot heat) above air temperatures (Greenberg & Kunich, 2002). In this study, larvae of large population sizes at 20 °C aggregated consequently generated temperatures over 40 °C and created a temperature gradient of ∼20 °C in the substrate (Fig. 8). An elevation of ∼20 °C above air temperature was also recorded in maggot heat generated by blow fly larvae, *Calliphora vomitoria* L. (Diptera: Calliphoridae) (Turner & Howard, 1992). It should be noted that the hottest temperatures did not increase with increasing air temperature (i.e., in the 20 °C vs. 30 °C treatments). Maximum temperatures generated were similar between large population size groups at both 20 and 30 °C treatments (Fig. 8, Fig. S6). Animal metabolic rates usually increased with increasing temperature, which is also true for black soldier flies (Gligorescu *et al.*, 2018). Theoretically, larvae in treatment 20L should have similar feed digestion rate, feed assimilation rate, and growth rate compared to 30L as long as they were in the hottest spot in the pan, meaning even though the optimal temperature for black soldier fly larval growth was determined around 30 °C (Chia *et al.*, 2018), industrial mass rearing should still work at lower temperature (e.g., ∼20 °C) as long as larvae start to aggregate and generate heat.

Thermoregulation allows organisms to maximize net energy gain (Angilletta, 2009). Larvae in the 20L treatment had similar AD but better FCR and ECD compared to larvae in the 30L treatment, indicating less assimilated energy might be allocated for basal metabolism and greater net energy was gained by larvae in 20L. The substrate temperatures were more evenly distributed across the pan at 30 °C (Fig. S5) compared to larvae reared at 20 °C, indicating the black soldier fly larvae may spread

out when the substrate temperature was high. Therefore, one could consider decreasing the unnecessary larval basal metabolism to maximize the larval weight gain in insect farming, though decreasing metabolic heat may not be ideal for those needing the extra metabolic heat for substrate water evaporation. The heat generated by high densities black soldier fly larvae (e.g., 6 larvae/cm^2) enables water evaporation, which is desirable for waste management purpose, and allow easier larval separation (Ermolaev *et al.*, 2020).

Hotter is smaller is a well-known temperature-size rule describing the general phenomenon that individuals developing at hotter temperature tend to be smaller (Angilletta & Dunham, 2003). However, as with most rules, there are exceptions to the temperature-size rule. For example, in this study the smallest larvae were observed in treatment 20S (e.g., small population size at 20 °C). The greatest FCR (Fig. 5), lowest ECD (Fig. 6), and lowest AD (Fig. 7) of treatment 20S indicate larvae could not digest and assimilate the feed effectively. Higher temperatures may be required for the optimum function of black soldier fly larval digestion. At least, enzyme activities for the protease and trypsin-like protease in BSF larvae have been determined to be around 47 °C (Kim *et al.*, 2011). Larvae in treatment 30S had a relatively low AD while maintained a relatively high ECD and average larval weight indicating beside temperature, other factors (e.g., enzymes secreted outside the body or purely physical property) could hamper feed consumption at low population size. Some larvae eat with the help of extra-oral digestive enzymes, which is more efficient in a group of hundreds and thousands of larvae (Descalzi, 2019).

To summarize, the substrate temperature is highly correlated with the larval population density, population size, and air temperature. By shifting the larvae from higher to lower air temperature along their development could increase larval yield while shorten rearing duration.

Conclusion

What is the optimum air temperature for black soldier fly larval rearing? The answer depends on a number of factors. The data in this study emphasized the importance of measuring larval substrate temperatures together with air temperatures. Substrate temperature can increase with the increments of larval population density, population size, and air temperatures. Larvae can benefit from maggot heat at low air temperature through larval activities. Such remarkable behavioral thermoregulation allowed insect energy gain and storage to be maximized in a heterogeneous thermal environment (e.g., the substrate temperature in 20L). Larvae can suffer at high air temperature when they cannot get rid of the heat by behavioral dispersion effectively (Fig. S7), which is more likely to occur in a thermally homogenous environment (e.g., the substrate temperature in 30L). If true, starting the rearing process at 30 °C and gradually shifting to lower temperatures when larvae get older could be a promising strategy for production optimization. A precise temperature management has proved to be essential for optimal production in other industries [e.g., poultry (Mesquita *et al.*, 2021) and silkworm *Bombyx mori* (L.) (Lepidoptera: Bombycidae) (Rahmathulla, 2012)].

Industrial scale results are different than benchtop studies (Yang & Tomberlin, 2020). More studies are needed in the future to understand such differences in between large- and small-scale operations. For example, data in this study determined substantial heat was generated in pans even without larvae indicating microbes in the substrate may contribute significantly to the substrate temperature in addition to larval metabolism and movement. Future studies ought to integrate more variables and measure the metabolic heat production of larvae directly. Finally, these results are population specific since genetic structures differ among populations (Kaya *et al.*, 2021). Producers should determine the critical temperatures for their fly populations before applying specific temperature treatments.

Disclosure

The authors declare no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Heat management experimental setup with different black soldier fly population size (L, M, and S for 10 000, 1 000, and 166 larvae) and air temperature (20 and 30 °C).

Fig. S2 The daily maximum temperature (Mean ± CI) in substrates with different black soldier fly larval density (0–10 000 larvae/pan with 8 kg diet) placed in a walk-in chamber under 27.0 ± 0.5 °C, 60% ± 5.1% RH, 14 : 10 L : D.

Fig. S3 The daily temperature variation (Mean ± CI) in substrates with different black soldier fly larval density (0–10 000 larvae/pan with 8 kg diet) placed in a walk-in chamber under 27.0 ±0.5 °C, 60% ± 5.1% RH, 14 : 10 L : D.

Fig. S4 The daily maximum temperature in substrate with different black soldier fly larval density (0–10 000 larvae/pan with 8 kg diet) differed from control (group = 0) in a walk-in chamber under 27.0 ± 0.5 °C, 60% ± 5.1% RH, 14 : 10 L : D.

Fig. S5 Surface temperature among treatments (i.e., large, median, and small size containers at either 20 or 30 °C) on day six in heat management experiment.

Fig. S6 The daily maximum temperature (Mean ± CI) in substrate with different black soldier fly larval population size (L, M, and S for 10 000, 1 000, and 166 larvae) and air temperature (20 and 30 °C).

Fig. S7 Temperature variation in pan due to larval aggregation.