Environmental Entomology, XX(X), 2017, 1–9
doi: 10.1093/ee/nvx165
Research

Photophase Duration Affects Immature Black Soldier Fly (Diptera: Stratiomyidae) Development

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Subject Editor: Colin Brent

Received 16 July 2017; Editorial decision 21 September 2017

Abstract

This study tested the effect of photophase duration on black soldier fly, *Hermetia illucens* (L.; Diptera: Stratiomyidae), development. Successful larval eclosion, development time and adult emergence were measured for individuals exposed to 0 h, 8 h, and 12 h of light, at approximately 27°C and 70% relative humidity. Accumulated degree hours (ADH) were calculated to correct for differences in temperature across treatments. Larvae successfully eclosed in all treatments, with larvae in 12 h light requiring 5.77% and 4.5% fewer ADH to eclose than larvae in 0 h and 8 h, respectively. Overall, larvae in 0 h required 39.34% and 37.78% more ADH to complete their development from egg to adult than larvae in 8 h and 12 h, respectively. The effect of photophase duration on juvenile development was largest in the post-feeding stage, and smallest in the pupal stage. Specifically, post-feeding larvae in 0 h required 80.02% and 90.08% more ADH to pupate than larvae in 8 h and 12 h, respectively, but pupae in 8 h required 9.63% and 7.52% fewer ADH to eclose than pupae in 0 h and 12 h, respectively. Lastly, larval mortality was significantly higher in 0 h, with 72% survivorship, and 96% and 97% in 8 h and 12 h, respectively. However, 17.8% of mortality in the absence of light is hypothesized to be a result of predation by Arachnidae and Blattidae. These data could prove valuable for optimizing industrial processes for mass-production of this species for use as alternative protein in feed for livestock, poultry, and aquaculture.

Key words: aquaculture, photoperiodism, waste conversion, *Hermetia illucens*, insect-based protein production

For insect mass-rearing facilities, environmental optimization year around is essential to maintain maximized and predictable production. Aside from diet constraints, temperature, relative humidity (RH) and day length are three primary factors taken into consideration when artificially rearing insects in mass production (Schneider 2009). Deciphering the constraints different abiotic factors have on a species' development will help ensure optimal production and minimize unnecessary costs in mass rearing facilities.

Insect development is regulated at two time scales, with internal circadian clocks tracking their environment on a 24-h scale, and photoperiodism tracking their environment on a 12-mo seasonal scale (Bradshaw and Holzapfel 2010). Internal circadian clocks enable insects to regulate behavioral and physiological processes with the use of photoperiods and/or thermoperiods (Saunders 2009). On longer time scales, photoperiodism enables insects to prepare for stressful environmental conditions using the shift in the length of light and dark periods throughout the year, where shorter days signify the onset of winter (Bradshaw et al. 2003).

Environmental stresses impact arthropod behavior and physiology. In some cases, arthropods migrate to more favorable conditions (Bale and Hayward 2010) or enter a state of quiescence, which is the direct inhibition of development due to abiotic conditions above or below developmental thresholds tolerated by cold shock or heat shock proteins (Hodek 2002). In other instances, an arthropod could enter diapause in response to seasonal cues, which suppresses development until more favorable conditions are present (Hodek 2002). It is important to avoid these environmental stresses in mass rearing facilities, as the objective of mass rearing is to produce high quality insects *en* mass as efficiently, and cost-effectively as possible.

Due to their ability to convert organic waste into 40% protein and 30% fat (Newton et al. 1977), the black soldier fly, *Hermetia illucens* (L.; Diptera: Stratiomyidae) has recently been used in mass-rearing facilities for producing protein for supplementation in feeds for livestock, poultry and aquaculture (see reviews Makkar et al. 2014, Sánchez-Muros et al. 2014, Henry et al. 2015). However, aside from light requirements for mating, including minimum light intensity (Tomberlin and Sheppard 2002, Zhang et al. 2010), and spectral light composition (Oonincx et al. 2016), where it is argued that light intensity plays a secondary role to light spectral composition (Oonincx et al. 2016), light duration requirements for optimal black soldier fly development are not known.

Evidence of diapause in the family Stratiomyidae is very limited with only one publication found (Rozkosny and Kovac 1998). Stratiomyids typically inhabit tropical regions (Woodley 2001); however, it is not known if *H. illucens* inhabiting temperate regions during the summer months are diapausing or migrating south for the winter. Due to short adult lifespans of just a few days to a few weeks (Tomberlin et al. 2002), it is more likely black soldier flies overwinter. Furthermore, if diapause is involved, the abiotic factors serving as cues to induce diapause are not known. The primary objective of this study was to determine the effect(s) photophase duration have on *H. illucens* development. Doing so will add to the growing body of literature (Bondari and Sheppard 1981; Bondari and Sheppard 1987; Larde 1989; Tomberlin et al. 2002, 2009; Myers et al. 2008; Diener et al. 2009; Gobbi et al. 2013; Holmes et al. 2012, 2013, 2016; Zhou et al. 2013; Yu et al. 2014; Nguyen et al. 2013, 2015; Lalander et al. 2015) on the rearing requirements of the black soldier fly. Because this species is a recognized candidate model for refining protein *en* mass from organic waste, knowing the effect(s) photophase duration have on black soldier fly development could prove crucial for optimizing its use in waste management and bioremediation efforts in temperate climates; efforts that could reduce global demands on an already overtaxed environment (Tomberlin et al. 2015).

Materials and Methods

Black Soldier Fly Colony

Eggs were collected from a black soldier fly colony housed in a screen mesh cage (1.8 × 1.8 × 1.8 m with 1.5 mm mesh) maintained in a greenhouse, outside the Southern Plains Agricultural Research Center (SPARC, College Station, TX) in February 2010. The colony was established in the spring of 2009 from the eggs of a laboratory colony initiated at the Coastal Plain Experiment Station, University of Georgia, Tifton, GA, which originated from a poultry facility in Bacon Co., GA in 1998. Eggs were collected over a 4 h oviposition window from females ovipositing in the 3 × 4 mm flutes (internal tubular spaces) of three-layer stacks of 0.5 (d) × 3 (w) × 5 (l) cm corrugated cardboard rectangles bonded together with glue. The stacks were constructed and mounted with the flutes oriented vertically, 5 cm above an oviposition attractant. Oviposition attractant (also used as the larval diet) was composed of moist-to-liquefied Gainesville diet (5:3:2 hand prepared mixture of wheat bran, alfalfa and corn meal, respectively); (Producers Cooperative Association, Bryan, TX), developed for rearing house flies, *Musca domestica* (L.; Diptera: Muscidae) (Hogsette 1992, Tomberlin et al. 2002).

Larval Eclosion

Each egg-containing corrugated cardboard flute was dissected to remove egg clutches. Individual egg clutches were randomly placed into 30 ml clear plastic soufflé cups (Dart P100, Dart Container Corporation, Mason, MI). Cups containing a single egg clutch (contribution from one female; flutes with more than one clutch were not used) were divided among three light treatments (0 h, 8 h, and 12 h of daylight). Ten replicate egg clutches for each treatment were placed in a 30-well tray (Bio-Serv, Frenchtown, NJ) and stored on the middle shelf of a growth chamber (Percival Scientific Inc. Model I-36LLVLC8, Perry, IA) with temperature, RH, and fluorescent photoperiod set to 27°C, 70% RH, and either 0:24, 08:16, or 12:12 (L:D). Photophase durations of 0 h, 8 h, and 12 h were chosen based on three considerations. First, a 12-h day length in Georgia is long enough to sustain successful rearing of the black soldier fly (Sheppard et al. 2002). Second, an 8-h day length represents the shorter days of the year in southern Canada, where several insect species experience hibernal diapause. Third, continuous darkness was chosen to determine if *H. illucens* development from egg to adult requires light due the effects of potentially limited light exposure in mass rearing facilities.

The growth chamber housing the 0-h daylight treatment was externally modified with a 1.22 × 1.83 × 2.13 m three-sided tent to prevent light contamination when opening the growth chamber door during experimental observations and data collection. The tent frame was crafted using one inch PVC piping and three way PVC connectors (Home Depot, College Station, TX). The tent walls were constructed using black and white poly plastic (Discount Garden Supply, Riverside, CA). The plastic walls and ceiling were taped together over the PVC frame with black Gorilla Brand duct tape and white 3M Brand duct tape (Home Depot). Seams were taped from both sides to reduce light penetration. While one end of the tent allowed ingress through a light-tight sealable port, the other was attached to the growth chamber. The poly plastic was taped to the top, sides and bottom of the growth chamber, ensuring no ventilation vents were covered, allowing the chamber to protrude 30 cm inside the tent.

The tent was designed with the black side of the poly plastic on the inside of the tent, while the white side faced the outside of the tent to reflect light away from the enclosed growth chamber. The bottoms of the walls were tucked under the PVC frame and taped to the cement floor with black Gorilla Brand duct tape on the inside of the tent. With respect to entry into the tent, a double door design composed of two layers of the same poly plastic comprising the walls and ceiling of the tent was implemented such that the first external door could be sealed upon entry before complete entry through a second door into the enclosed tent housing the growth chamber. LED lights on the front of the growth chamber were also covered in poly plastic. Night vision goggles (Eyeclops 2.0, Jakks Pacific, Malibu, CA) were used in stealth mode (no visible light emitted) to make observations and to record data in the 0:24 (L:D) treatment.

Eggs were monitored every 12 h for the first 3 d and then hourly until egg hatch. Elapsed time to larval eclosion was recorded from 30 min after females were given access to flutes, until the first hourly observation where egg hatch was observed. Two Hobo U12-012 data loggers (Onset Computer Cooperation, Bourne, MA) were placed in each growth chamber; one on the top shelf and one on the bottom shelf, to record RH, temperature and light intensity variation within growth chambers and among growth chambers. The data logger on the top shelf also had an external temperature probe which recorded temperature on the middle shelf. Temperature, RH and light intensity was recorded every 15 min and mean (±SEM) values were calculated for each from the pooled top, middle, and bottom shelf recorded data.

Feeding and Post-Feeding Treatment Effects

Eclosed larvae were used to determine the effects of day length on feeding and post-feeding larval development, and adult survivorship. Eclosed larvae remained in their respective lighting treatments. Upon egg hatch, each of the 10 replicate 30 ml cups containing a single egg clutch with eclosed larvae from each treatment was placed inside a small clear plastic Ziploc dish. Inside each of these dishes, 10 g aliquots of Gainesville diet; used previously as an oviposition attractant (Hogsette 1992, Tomberlin et al. 2002), mixed with 18 ml of water (70% moisture, Tomberlin et al. 2002) was added to one end of the dish. Hatched larvae migrated from their 30 ml cup to the food. Water was added as required to dried feed to maintain

moisture. Larvae were fed ad libitum for the first 6 d of development. The 30 ml cups containing hatched egg clutches were removed 2 d after larval eclosion and stored in a −20°C freezer.

The sample size of each replicate within each treatment was reduced to a manageable number of 50 individuals per replicate, per treatment, by blindly hand-selecting 50 six-day-old larvae and placing them in 2.2 L clear plastic Ziploc containers for an N = 1,500. Feeding larvae were provided 10 g of dry Gainesville diet mixed with 18 ml of water ad libitum and all individuals were monitored daily for ecdysis to post-feeding (i.e., prepupae, Sheppard and Newton 1994), pupation and adult eclosion. Upon reaching the post-feeding stage of development, each post-feeding larva was placed into a 30 ml clear plastic soufflé cup and labeled individually, such that each replicate still comprised 50 individuals (N = 1,500), but that these individuals no longer directly interacted with one another. Isolated individuals in their cups were placed into 30-well clear plastic trays and returned to their respective growth chamber and monitored daily for pupation. Cups housing pupated larvae were capped with a clear plastic lid (100PCL25 Dart Container Corporation, Mason, MI) and pierced with a 29-gauge syringe needle. Observations continued daily to record adult eclosion. Upon adult eclosion, individual adults were sexed and monitored daily until death. All observations, feeding and sampling in the 0-h daylight treatment was done within the confines of the poly plastic tent, preventing exposure to visible light rays. Mean (±SEM) elapsed time in the feeding, post-feeding, and pupal stages of development, successful adult emergence, adult longevity and number of virgin females to oviposit were recorded.

Statistics

All statistics were computed using RStudio statistical software (R Development Core Team 2016, version 0.99.893).

Abiotic Factors

Mean (±SEM) temperature, RH, and light intensity, was compared across daylight exposures to test for treatment and growth chamber effects. Temperature and RH were analyzed independently assuming a generalized linear model with a Gaussian distribution. After conducting a Box-Cox analysis, RH data was squared prior to analysis. Significant differences were considered for P value < 0.05. Differences were identified using the function general linear hypotheses test (glht) (multcomp package, Hothorn et al. 2008).

Survivorship

Survival from 6-d-old larva to adult eclosion was analyzed using a generalized linear model with a binomial distribution. Significant differences were identified assuming the ANOVA function with a Chi-square test (P < 0.05). Post hoc analysis was done using the function glht (multcomp package, Hothorn et al. 2008) and P values were adjusted for type I error using the Bonferroni method.

Development Time

Despite setting growth chambers to 27°C across all treatments, the Hobo data loggers revealed statistically significant differences in temperature across treatments, and therefore development time data for each stage of development was converted to accumulated degree hours (ADH) prior to analyses. ADH were calculated using the following equation:

$$\mathrm{ADH} = \sum_{j=1}^{24} (\theta_{o,j} - \theta_b)_{(\theta_{o,j} - \theta_b) > 0)}$$

Where $\theta_{o,j}$ is the mean temperature recorded across all data loggers for hour j and θ_b is the base temperature where growth and development does not occur. In addition, where $\left((\theta_{o.i} - \theta_b) < 0\right)$ the value of $(\theta_{o,j} - \theta_b)$ was set to zero for that hour. A base temperature θ_b, of 16°C was used in all ADH calculations which was determined experimentally in Holmes et al. (2016), where eggs survived and successfully hatched in 16°C, but suffered 100% mortality within 3 d of hatching. Since temperature was determined to not significantly influence ADH requirements for egg hatch (Harnden and Tomberlin 2016), a single base temperature was used in all ADH calculations.

Time to egg hatch data were bimodal. After observing that the first peak in the histogram was comprised entirely of one treatment and the second peak comprised the other two treatments combined, it was decided that the mean (±SEM) time to larval eclosion be analyzed with a non-parametric one-way Wilcoxon/Kruskal-Wallis (Ranked Sums) analysis. Post hoc analyses using Dunn's Kruskal-Wallis multiple comparisons test (dunnTest) was used on significant differences (P < 0.05) (dunn.test package, Dinno 2016). P values were adjusted using the Bonferroni method to correct for type I error. Differences were considered significant with P values < 0.05.

Mean (±SEM) feeding, post-feeding and pupal development times, and adult longevities were analyzed independently assuming a generalized linear model with a Gaussian distribution to test for length of photoperiod effects, sex and the interaction of photoperiod and sex on length of larval development time for feeding, post-feeding and pupal stages. Post-feeding development time and total development time was transformed by taking the inverse (x^{-1}) and $(x^{-2.5})$, respectively after completing a Box-Cox analysis. Pupal development time was log transformed. Post hoc general linear hypotheses and multiple comparisons were completed on significant results (P < 0.05) using the function glht (multcomp package, Hothorn et al. 2008). P values were adjusted using the Bonferroni method to correct for type I error.

Virgin Female Oviposition

The number of virgin females to oviposit was analyzed assuming a generalized linear model with a binomial distribution. Significant differences were identified assuming the ANOVA function with a Chi-square test (P < 0.05). Post hoc analysis was done using the function glht (multcomp package, Hothorn et al. 2008) and P values were adjusted for type I error using the Bonferroni method.

Results

Abiotic Factors

Temperature and RH varied across treatments (glm: $F_{2,8710}$ = 12741, P < 0.001 and $F_{2,19152}$ = 1327.5, P < 0.001, respectively, see Table 1). The 0 h of daylight treatment was 0.88°C and 1.99°C lower in temperature and 2.48 and 1.07% lower in RH compared to the 8 h (glht: z = 146.66, df = 2, P < 0.001 and z = 22.01, df = 2, P < 0.001, respectively), and 12 h (glht: z = 94.45, df = 2, P < 0.001 and z = 51.41, df = 2, P < 0.001, respectively) daylight treatments. The 12 h of daylight treatment had a higher mean temperature, but a lower mean RH than the 8-h treatment (glht: z = −39.68, df = 2, P < 0.001 and z = 28.01, df = 2, P < 0.001, respectively). Lastly, as a result of different day lengths in each treatment, light intensity measured in Lux was also different across treatments (χ^2 = 14427.22, df = 2, P < 0.001). Specifically, the 0-h treatment had the lowest light intensity compared to the 12-h and 8-h day lengths (Dunn Test: Z = −114.43, P < 0.001 and Z = −87.57, P < 0.001, respectively), and the 8-h day length light intensity was lower than the 12-h treatment (Dunn Test: Z = 29.23, P < 0.001, Table 1).

Table 1. Mean (±SEM) hobo data logger outputs for temperature, relative humidity and light intensity from each day length treatment (0 h, 8 h, and 12 h)

Day length (h)	Temperature (°C)			Relative humidity (% RH)			Light intensity (Lux)		
	Daylight	Dark	Mean	Daylight	Dark	Mean	Daylight	Dark	Mean
0	26.35 ± 0.00	26.35 ± 0.00	26.35 ± 0.00a	72.35 ± 0.01	72.35 ± 0.01	72.35 ± 0.01a	2.49 ± 0.02	2.49 ± 0.02	2.49 ± 0.02a
8	28.04 ± 0.01	26.80 ± 0.00	27.23 ± 0.00b	72.10 ± 0.08	76.26 ± 0.03	74.83 ± 0.02c	4215.90 ± 16.64	11.94 ± 0.01	1456.51 ± 14.81b
12	28.78 ± 0.01	27.88 ± 0.01	28.34 ± 0.00c	71.73 ± 0.07	75.19 ± 0.04	73.42 ± 0.02b	5650.37 ± 16.48	13.58 ± 0.04	2905.07 ± 20.90c

Means followed by different letters within the same column are significantly different ($P < 0.001$). Mean column for each abiotic factor is the mean recorded values pooled from data loggers placed on the top, middle, and bottom shelves of the growth chamber across both daylight and dark hours of the experiment.

Larval Eclosion

The ADH required for larval eclosion differed between day lengths (χ^2 = 17.602, df = 2, $P < 0.001$, Table 2). Larvae in the 12-h (944.527 ± 6.75 ADH) treatment required 4.50% and 5.77% fewer ADH to eclose than larvae in the 8-h (988.06 ± 7.22 ADH) and 0-h (1000.64 ± 2.74) treatments (Dunn test: $Z = 2.88$, $P = 0.012$ and $Z = 4.08$, $P < 0.001$, respectively). Larval eclosion in the 8-h treatment did not differ from larval eclosion in the 0-h treatment (Dunn test: $Z = 1.20$, $P = 0.692$).

Feeding and Post-Feeding Development

Upon hatching, the required ADH to complete the feeding stage of development differed with treatment (glm: $F_{2,1232} = 162.33$, $P < 0.001$) and sex (glm: $F_{1,1231} = 33.23$, $P < 0.001$), with no interaction (glm: $F_{2,1229} = 0.24$, $P = 0.918$). Specifically, larvae the 0-h day length treatment (4828.68 ± 26.11 ADH) accumulated 11.82% more degree hours than larvae in 8 h (4289.92 ± 22.14 ADH) (glht: $z = -16.67$, $P < 0.001$), which required 8.27% fewer degree hours than larvae in the 12 h treatment (4659.84 ± 16.86 ADH) to complete the feeding stage of development (glht: $z = -12.09$, $P < 0.001$), regardless of sex (Table 2). In addition, females (4678.28 ± 23.05 ADH) required 4.90% more ADH than males (4454.73 ± 22.02 ADH) to complete the feeding stage of development, regardless of day length treatment (glht: $z = -8.16$, $P < 0.001$).

Although there was no treatment by sex interaction on the required ADH to complete the post feeding stage of development (glm: $F_{2,1300} = 1.92$, $P = 0.146$), both main effects of treatment (glm: $F_{2,1303} = 635.94$, $P < 0.001$) and sex (glm: $F_{1,1302} = 28.70$, $P < 0.001$) influenced the number of ADH required to complete the post-feeding stage of development (Table 2). Specifically, post-feeding larvae in the 0-h treatment (6770.37 ± 165.18 ADH) required 80.02% and 90.08% more ADH to pupate than those in 8-h (2900.72 ± 49.47 ADH, glht: $z = 26.58$, $P < 0.001$) and 12-h (2565.55 ± 56.44 ADH, glht: $z = 34.79$, $P < 0.001$) treatments, respectively. Similarly, post-feeding larvae in the 8-h treatment required 12.26% more ADH to pupate than those in the 12-h treatment (glht: $z = -8.92$, $P < 0.001$), regardless of sex. Additionally, males (3958.71 ± 71.59 ADH) required 8.57% more ADH to pupate than females (3633.38 ± 70.72 ADH) (glht: $z = -5.35$, $P < 0.001$), regardless of treatment.

ADH required in the pupal stage of development to complete metamorphosis differed by day length treatment (glm: $F_{2,1300} = 32.20$, $P < 0.001$) and sex (glm: $F_{1,1300} = 11.46$, $P = 0.001$), with no interaction (glm: $F_{2,1300} = 0.53$, $P = 0.587$) (Table 2). Pupae in 0-h (2325.27 ± 33.60 ADH) and 12-h (2276.55 ± 14.99 ADH) day length treatments did not differ in the number of ADH required to complete metamorphosis ($P = 0.677$). However, pupae in the 8-h (2111.65 ± 16.92 ADH) treatment required 9.63% and 7.52% fewer ADH to complete metamorphosis compared to pupae in 0 h (glht: $z = -7.08$, $P < 0.001$) and 12 h (glht: $z = -6.13$, $P < 0.001$), respectively. Males (2188.89 ± 10.60 ADH) required 3.52% fewer ADH to complete metamorphosis than females (2267.28 ± 13.81 ADH) (glht: $z = -3.26$, $P = 0.004$).

Overall, the total ADH required to complete development from egg to adult was different across treatments (glm: $F_{2,1303} = 750.00$, $P < 0.001$). Specifically, larvae in 0 h required 39.34% (glht: $z = 37.20$, $P < 0.001$) and 37.78% (glht: $z = 34.08$, $P < 0.001$) more ADH than larvae in 8 h and 12 h, respectively (Table 2). Additionally, black soldier fly reared in 8 h of daylight required 1.61% (glht: $z = 3.46$, $P = 0.002$) fewer ADH than black soldier fly reared in 12 h of daylight (Fig. 1, Table 2).

Table 2. Mean (±SEM) length of black soldier fly development by life-stage and percent successful adult emergence across day lengths. Development time is presented in days and ADH (to correct for differences in temperature across treatments, see Table 1)

Day length (h)		Egg stage	Feeding larval stage	Post-feeding larval stage	Pupal stage	Total development (egg to adult)	Successful adult emergence (%)[a]		Adult longevity
0	Days	3.72 ± 0.02	18.33 ± 0.12	29.62 ± 0.70	9.66 ± 0.14	66.37 ± 0.11	72.2 ± 0.02c	Females	10.18 ± 0.19
								Males	14.34 ± 0.39
	ADH	1000.64 ± 2.74a	4828.68 ± 26.11a	6770.37 ± 165.18 a	2325.27 ± 33.60a	15329.89 ± 193.76a		Females	2446.96 ± 51.83ab
								Males	3474.87 ± 97.04c
8	Days	3.68 ± 0.03	15.70 ± 0.11	11.10 ± 0.19	8.38 ± 0.07	47.49 ± 0.07	95.8 ± 0.01b	Females	10.03 ± 0.14
								Males	11.75 ± 0.19
	ADH	988.06 ± 7.22a	4289.92 ± 22.14c	2900.72 ± 49.47 b	2111.65 ± 16.92b	10290.10 ± 56.63c		Females	2605.20 ± 39.05b
								Males	3058.41 ± 51.10 d
12	Days	3.20 ± 0.02	15.35 ± 0.11	9.47 ± 0.20	8.41 ± 0.05	41.05 ± 0.07	96.8 ± 0.01a	Females	8.61 ± 0.14
								Males	9.71 ± 0.20
	ADH	944.52 ± 6.75b	4659.84 ± 16.86b	2565.55 ± 56.44 c	2276.55 ± 14.99a	10457.43 ± 55.20b		Females	2295.81 ± 39.04a
								Males	2632.17 ± 54.93b

All statistical comparisons were done on ADH development data. Different letters within the same column required significantly different ADH to complete each stage ($P < 0.001$).

[a]Means for successful adult emergence followed by different letters are significantly different ($P < 0.001$).

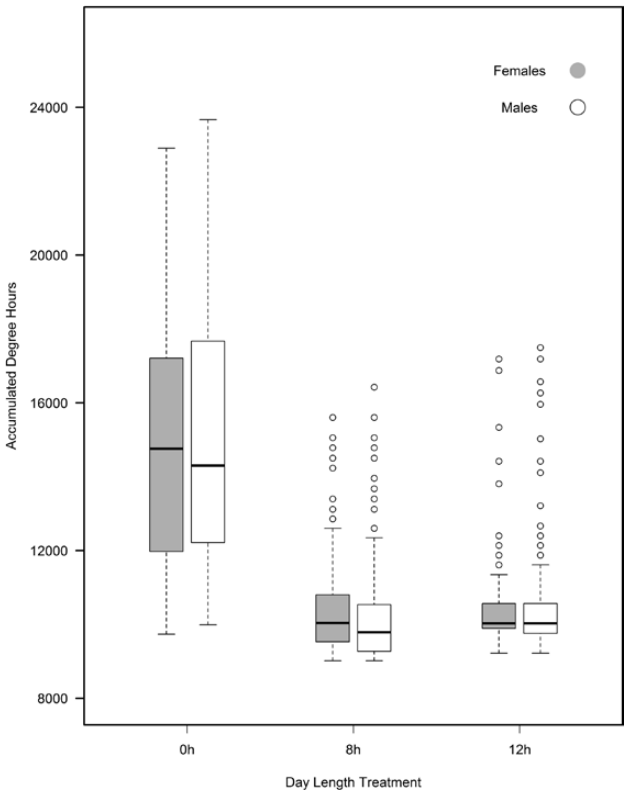

Fig. 1. Boxplot distribution of total development time for black soldier fly immatures measured in ADH across day length treatments 0 h, 8 h, and 12 h, at 27°C and 70% RH, respectively. The horizontal line within the box indicates the median, the lower and upper boundaries of the box indicate the 25th and 75th percentiles, respectively, and the upper and lower dashed lines protruding from each box are the highest and lowest values, respectively, of adult longevity. Females are represented in grey, males in white for each treatment. Regardless of sex, the ADH required to reach the adult stage of development increased with decreasing daylight exposure, boxplots with different letters are significantly different ($P < 0.001$).

Survivorship

Overall successful adult eclosion differed between treatments ($\chi^2 = 177.80$, df = 2 $P < 0.001$) (Table 2). The 0-h (72.2%) treatment had lower successful adult emergence than both 8 h (95.8%, glht: $z = 8.90$, $P < 0.001$) and 12 h (96.8%, glht: $z = 8.99$, $P < 0.001$). There was no difference in adult eclosion success between 8 h and 12 h ($P = 1.000$). Furthermore, there was no evidence of a sex bias among treatments ($\chi^2 = 0.39$, df = 2, $P = 0.823$) with 51.31%, 49.90% and 51.86% females for each treatment, 0 h, 8 h, and 12 h, respectively. It's noteworthy to mention here that of the 27.8% of larval mortality in the absence of light, 17.8% comprises missing larvae hypothesized to have been predated by spiders and cockroaches, discussed later.

Adult longevity differed in response to an interaction between day length and sex (glm: $F_{2,1299} = 20.26$, $P < 0.001$) (Table 2). While ADH decreased with increasing day length (0 h, 8 h, and 12 h) for males ($P < 0.001$) (12.75% and 27.60%, respectively), females in the 0-h treatment did not differ from females in 8 h ($P = 0.351$) or 12 h ($P = 0.392$), nor did they differ from males in 12 h ($P = 0.191$). However, females in the 8-h day length treatment had 12.63% more ADH than females in 12 h ($P < 0.001$), but did not differ from males in 12 h ($P = 0.999$) (Fig. 2).

The percent of virgin females to lay eggs in the 0 h, 8 h, and 12 h of daylight treatments ($N = 178$, 239, and 251, respectively),

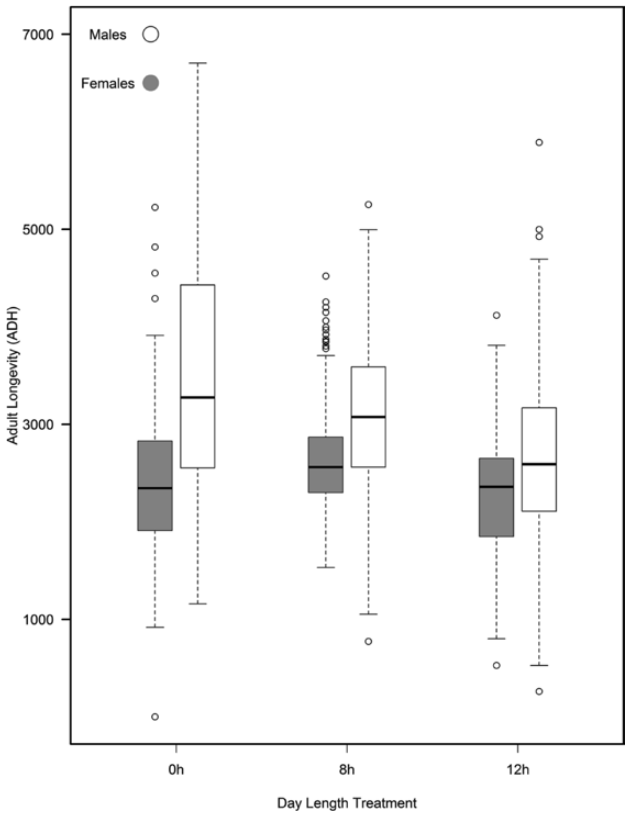

Fig. 2. Boxplot distribution of black soldier fly adult longevity across day length treatments 0 h, 8 h, and 12 h, at 27°C and 70% RH, respectively. The horizontal line within the box indicates the median, the lower and upper boundaries of the box indicate the 25th and 75th percentiles, respectively, and the upper and lower dashed lines protruding from each box are the highest and lowest values, respectively, of adult longevity. Females are represented in grey, males in white for each treatment. The interaction of treatment and sex for adult longevity measured in ADH was significant, where boxplots with different letters are significantly different ($P < 0.001$).

was 25.28%, 19.67%, and 11.16%, respectively. This difference was significantly different (glm: $\chi^2 = 15.62$, df = 2, $P = 0.001$) across treatments. More virgin females reared in complete darkness and 8 h oviposited than those reared in 12 h of daylight (glht: $z = -3.80$, df = 1, $P < 0.001$ and glht: $z = 2.58$, df = 1, $P = 0.026$, respectively). However, the number of virgin females to oviposit did not differ between the 0 h and 8 h of daylight treatments ($P = 0.326$).

Discussion

Shorter days often induce hibernal diapause (dormancy) among most temperate arthropods (Bradshaw and Holzapfel 2001). Although it is not known if the absence of light is detrimental to black soldier fly development, our findings indicate a significant delay in overall development in its absence due primarily to a substantial increase in the duration of the post-feeding larval stage (Fig. 2, Table 2). While feeding times are often prolonged in insects experiencing pre-hibernal diapause (Matsunaga et al. 1995), this does not completely explain the extended feeding time for black soldier fly larvae in the absence of light. Specifically, post-feeding larvae experienced a greater delay than feeding larvae, and upon pupating, adult eclosion was delayed only a day (Table 2), suggesting that hibernal diapause may have been evident in the post-feeding stage instead. However, with environmental conditions static throughout the experiment, it is not clear what signaled adult eclosion in the absence of light.

Our findings also suggest that the length of the photophase exposure may also be important for black soldier fly development, as larvae required fewer ADH to complete development when exposed to shorter daylengths 8:16 (L:D) than those in longer daylengths 12:12 (L:D) (Table 2). The influence of photoperiod on the thermal requirements for insect development was first demonstrated by Lopatina et al. (2007), where linden-bug, *Pyrrhocoris apterus* (L.; Heteroptera: Pyrrhocoridae) development is less temperature dependent under short photophases compared to long photophases. Specifically, under shorter days, linden-bugs have a lower thermal requirement than under longer days, presumably to allow the insect to develop to its adult stage where it can survive the winter (Lopatina et al. 2007). This may explain the lower ADH requirements for black soldier fly larvae reared in 8 h compared to 12 h (Table 2), should black soldier flies undergo diapause, and should they undergo diapause in a later stage of development.

Other studies examining the effects of day length on insect development tend to observe one of two things: 1) insects develop faster at decreasing photoperiods resulting in diapausal adults as occurs in the eight-toothed spruce bark beetle, *Ips typographus* (L; Coleoptera: Scolytidae) (Dolezal, Habustová, and Sehnal 2007); or 2) insect development does not change when reared under decreased photoperiods and diapause is not induced such as in *Orius niger* (Wolff; Hemiptera: Anthocoridae) (Bahsi and Tunc 2008). However, these findings can vary drastically depending on the experimental design. Specifically, experimental designs aiming to test diapause induction by reducing day length are less likely to succeed if the reduction in day length is drastic and static (i.e., static photoperiods of 8 h vs 12 h of daylight), because the cue for diapause is believed to be the gradual lengthening of night (Kamm 1972). Generally, insects reared at a steady decrease in photophase duration (i.e., *x* number of minutes of daylight reduced per day) can stimulate diapause after a critical photoperiod is reached (Kamm 1972). Therefore, to determine whether diapause is a concern for black soldier fly reared in northern latitudes where day length varies seasonally, exposing them to a steady decrease in day length is a necessary next step for black soldier fly utilization for waste management and protein supplementation.

The extended time spent in the post-feeding stage of development in the absence of light, but similar development times spent in the pupal stage of development as in the presence of light is unclear. Specifically, if hibernal diapause was established in the feeding and post-feeding developmental stages, the cue for adult eclosion in the absence of light is unknown. However, it is possible that adult black soldier flies may be able to detect infrared light. For example, Shintani et al. (2009) found the carabid beetle, *Leptocarabus kumagaii* (Kimura & Komiya; Coleoptera: Carabidae), when exposed to white light after surgical removal of the compound eye, does not respond to photoperiodism. They also determined the stemmata in the immatures are responsible for responses to photoperiodism, but the stemmata derived organs in the adult brain are not necessary for photoperiodism (Shintani et al. 2009). Further, in the same study, Shintani et al. (2009) also found that some larvae did not respond to photoperiods of blue, green, or yellow light. So perhaps in *H. illucens*, larval stemmata also differ from the photoreception of the compound eye, and therefore, the photoreceptor spectral sensitivity may differ between juvenile and adult life stages.

Recently, Oonincx et al. (2016) measured the black soldier fly's photoreceptor spectral sensitivity in response to a range of wavelengths from 255 nm to 750 nm, and found that the compound eye of adults is most sensitive to short wavelengths in the ultraviolet and blue range, however, infrared ranges beyond 750 nm were not

tested. *H. illucens* mating can be partially stimulated by artificial lights (Tomberlin and Sheppard 2002, Zhang et al. 2010, Oonincx et al. 2016) and has been demonstrated to be unique in its photoreception compared to other taxa (Oonincx et al. 2016). As pupae complete metamorphosis and the photoreceptors in the compound eye develop, it is possible the infrared light rays emitted from the night vision goggles may serve as an emergence cue for the uneclosed adults.

Evidence of diapause in the family Stratiomyidae is very limited with only one published record indicating pupal diapause (Rozkosny and Kovac 1998). However, during the first attempt of rearing the black soldier fly in Windsor, Ontario in a greenhouse during the fall of 2008, *H. illucens* development was only successful to the pupal stage. When adults did not emerge more than a month after pupation, a new colony was initiated from eggs purchased from Dr. Craig Sheppard (University of Georgia, Tifton, GA); the original source of the colony in this study. However, approximately 3 mo after the original colony pupated, adult eclosion in the original colony was observed (personal observation, Holmes 2010). This period of dormancy in the pupal stage, is indicative of hibernal diapause. Since temperature in the greenhouse varies across seasons between 20–40°C (Holmes et al. 2013), it is not clear which indicators, temperature or photoperiod, or a combination of the two, cues diapause in the black soldier fly. However, in a laboratory study at 18°C (below that of the greenhouse housing the colony in the winter), while feeding and post-feeding instars were delayed, dormancy in the pupal stage was not observed (Holmes et al. 2016), suggesting photoperiod may play a role, or the interaction of temperature and photoperiod may be a critical combination.

It is not clear which life stage is susceptible to diapause cues. For example, whether adults receive the cue and diapause is induced in their offspring, or the larvae receive the cue and induce diapause in later developmental stages. Although, since the aforementioned new cohort of eggs provided from Craig Sheppared after adult eclosion was not observed in the greenhouse at the University of Windsor in 2008 were provided from Georgia, United States; where females that contributed the eggs would not have experienced environmental cues for diapause, it is likely the juvenile stages are susceptible to diapause cues, rather than the adults. Furthermore, based on the aforementioned personal observation, and the results of this study, it would appear that black soldier fly can enter a period of dormancy in the pupal and post-feeding stages of development, but further studies are needed to understand which abiotic factors cue diapause and which life stages are sensitive to diapause cues. Again, it is not clear if pupating black soldier fly can detect light at all, or what the photoreceptor spectral sensitivity is of feeding instars, and thus should be explored further.

Adult longevity increased with decreasing exposure to light, with adults living the longest in complete darkness. It was observed that adults reared in the presence of light were more active, flying around their cups the instant the lights turned on inside the growth chamber. On the other hand, adults reared in complete darkness were very docile throughout the duration of their adult life such that even with the disruption of tapping the cups to assess for mortality, the adults did not appear alarmed and instead of flying around the cup, would simply walk around the cup, likely exhausting fewer resources than adults in the light treatments that were often found attempting to fly.

Less than 1% of virgin females in Tomberlin et al. (2002) laid eggs, whereas 18% of virgin females laid eggs in this study. In stressful environments, female insects can resorb their eggs (Richards and King 1967), possibly to obtain nutrients, but the precise role of egg resorption is unclear (Rivero-Lynch and Godfray 1997). Evidence for egg resorption in the black soldier fly has never been documented, instead females typically develop a single clutch over their lifetime and in the absence of mating will oviposit infertile eggs (Tomberlin et al. 2002). Females that did not oviposit infertile eggs in this study may have delayed oocyte development and maturation in response to an absence of mating. Tomberlin et al. (2002) dissected virgin females 4 d after emergence and noted no mature eggs, whereas mated females dissected 2 d after mating had completely formed eggs. However, delaying oocyte development has been shown to reduce realized fecundity (Torres-Vial et al. 2002). While post-diapausing females often demonstrate reduced fecundity, feeding hibernal diapausing larvae can exhibit increased fecundity in post-diapausal females (Ishihara and Shimada 1995, Chang et al. 1996, Bradshaw et al. 1998). This may explain why more females in 0-h and 8-h oviposited infertile eggs compared to females in 12 h; however, since virgin females that did not oviposit were not dissected to assess reproductive status, any correlation (if any) between number of females to oviposit infertile eggs and potential fecundity is unknown. Furthermore, mating is not observed in light conditions below 63 µmol m^{-2} s^{-1} (3402 and 5166 Lux, for sunlight and fluorescent lighting, respectively) (Tomberlin and Sheppard 2002), and since we did not determine mating and oviposition success on adults reared at decreased hours of daylight (8 h), we cannot attest to higher fecundity in virgin female oviposition reared in 8 h and 0 h of daylight.

Our study revealed a higher proportion of mortality in the absence of light compared to daylight treatments. Here, we provide three possible hypotheses: 1) post-feeding larvae wandered excessively long periods before pupating in complete darkness and may have exhausted their fat resources and were unable to metamorphose; 2) related to our first hypothesis, post-feeding larvae may have suffered increased water-loss due to increased respiration as a response of excess wandering; 3) predation by Arachnidae and/or Blattidae, both were observed during daylight hours inside the light exclosure tent where the 0-h treatment growth chamber resided, but not in close proximity to the light treatments. However, Arachnidae and/or Blattidae were never observed inside any of the growth chambers during sampling times. In addition to 17.8% of post-feeding larvae simply disappearing from their cups in the 0-h daylight treatment over the course of the experiment, it was observed that dead post-feeding larvae collected were flattened and hollow; insinuating desiccation or removal of fat body. However, since mortalities in the daylight treatments were not dissected for comparison, the only evidence we have to support this hypothesis is the increased presence of Arachnidae and Blattidae inside the light exclosure tent and the disappearance of post-feeding larvae from their cups in the absence of light treatment. It is possible these post-feeding larvae simply escaped, however, a thorough search of the growth chamber and surrounding area did not reveal any post-feeding or pupal black soldier fly. In addition, larvae in the light treatments were housed in the same cups, but we did not observe missing individuals in these treatments, which would have experienced between 12 h and 16 h of night (absence of light), when predation risk is assumed to be higher by Arachnidae and Blattidae.

In summary, day length significantly affected *H. illucens* development. Although black soldier flies reared in complete darkness took several weeks longer, requiring several thousand more ADH to complete development than those reared in the presence of light, 72% did successfully emerge as adults. In addition, because the pupal stage of development did not differ more than a day and a half, the induction of diapause was not observed, but simply an incredibly long post-feeding stage of development in the absence of light.

After personal observations of diapause in black soldier fly rearing in Windsor, Ontario during the winter, it would be of interest to expose black soldier flies to a steady decrease in day length across a range of temperatures to truly assess the cues responsible for diapause in the black soldier fly. Personal observations and one published record (Rozkosny and Kovac 1998) indicate that black soldier flies diapause in the pupal stage of development and therefore we would not expect to see reproductive diapause in the adults, however post-diapause influences on adult life-history including fecundity, mating success and longevity are unknown and further investigation is recommended. Furthermore, longer feeding stages are often correlated with larger larval biomass, however, was not measured in this study, further investigations on waste conversion rates and biomass accumulation in the absence of light are needed. With this in mind, since the post-feeding stage of development was the stage most affected by the absence of light, a stage after which waste conversion has already occurred and where larval biomass is only expected to decrease with post-feeding energy expenditure, the mass rearing of black soldier fly for the purpose of harvesting post-feeding larvae for protein supplementation, black soldier fly can be reared in an enclosed vessel characteristic of complete darkness.

Acknowledgments

We would like to thank Craig Sheppard for supplying the black soldier fly larvae. We extend our warmest thanks to Longyu Zheng, Andrew Fields, Michelle Sanford, Micah Flores, Jennifer Pechal, Adrienne Brundage, Rachel Mohr and Eric Denemark for their help and support in building modifying the growth chamber, colony maintenance and laboratory operations. L.H. extends a special thanks to Oliver Love for his mentorship. We would also like to thank the Department of Entomology at Texas A&M University for hosting this research project and a special thanks to Tawni Crippen for providing use of greenhouse space at the USDA. Lastly, funding for this research was made possible by Natural Science and Engineering Research Council, Essex-Windsor Solid Waste Authority and Gesing Consultants Ltd.

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