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Growth Performance and Life History Traits of Black Soldier Fly (Hermetia illucens) Larvae Reared on Water Hyacinth – Based Substrates

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Abstract

Black Soldier Fly larvae (BSFL) have the potential to replace major feed ingredients in the diets of animals, including poultry, fish and pigs. This is due to their remarkable nutrient profile and efficiency in converting diverse waste streams into protein-rich biomass. The nutrient profile of BSFL is largely dependent on the nutrient composition of their rearing substrate. Therefore, the use of nutrient-dense substrates in the rearing process is highly crucial. In this study, BSFL were reared on eleven (11) water hyacinth–based substrates (water hyacinth combined with either milled para rubber seed, pig manure or fruit waste in distinct proportions) to determine the best feeding substrate that positively influenced larval growth. All analyses were performed using the R statistical software tool (version 4.4.3). The results revealed the most effective substrate to be a 50:50 ratio of water hyacinth and milled para rubber seed (PRWH-A). In addition, larvae reared on PRWH-A demonstrated the quickest development to prepupation (9 days), which was comparable to the control diet (Chicken feed). Similarly, the substrate that comprised all four organic wastes (WPSF) supported faster larval development (9 days) and outperformed the other substrates by recording the shortest time to 50% prepupation. Overall, feeding substrates formulated from distinct combinations of pig manure and water hyacinth demonstrated suboptimal performances, with the poorest outcomes recorded on SMWH-B (pig manure and water hyacinth in a ratio of 20:80).

Introduction

In recent years, there has been a notable increase in the use of Black Soldier Fly (*Hermetia illucens*) larvae (BSFL) in composting a wide spectrum of organic waste substrates like manure, food and municipal waste (da Silva and Hesselberg 2019). *Hermetia illucens* is considered a sustainable tool due to its remarkable efficiency in converting organic waste into protein-rich biomass (Tariq et al. 2025). Furthermore, they provide significant environmental benefits through efficient recycling of nutrients and low emissions of greenhouse gases during bioconversion processes (Arya et al. 2025). This aligns well with circular bioeconomy initiatives.

Studies show that BSFL has an excellent nutrient profile, comprising amino acids, minerals and fatty acids, which makes them a promising candidate for animal feed production (Iqbal et al. 2025). The incorporation of BSFL in animal feed formulation can help to increase animal yield, thereby increasing farmers' income. Shorter rearing cycles, zero-pathogen transmission properties, high crude protein and fat contents (42% and 29%, respectively), and the ability to thrive on various organic waste streams reinforce BSFL as the ideal component of insect-based animal feed (Wardhana et al. 2016; Wang & Shelomi, 2017). This is particularly relevant for Africa, which still grapples with food insecurity and food feed competition issues.

Although BSFL-derived animal feed has the potential to enhance overall growth performance in animals, rearing substrates play a vital role in the process. Given the substantial effects of substrate type on BSFL nutrient profiles, it is crucial to select optimal substrates to achieve larvae with desirable nutrient outcomes. As a result, most studies have focused solely on high-nutrient substrates. Over the years, kitchen and abattoir wastes, chicken feed, brewery and agro-industrial by-products and animal manures have been overutilised in BSFL production (Arshad et al., 2022; da Silva and Hesselberg 2019; Meneguz et al. 2018 ; Diola et al. 2024). In contrast, the potential of low-value, abundant plant biomass, particularly invasive

aquatic plants, remains underexplored. According to (Mufungwe et al. 2025), a major setback facing BSFL production in Africa is substrate profiling, which triggers the need to investigate more substrates to promote the safety and quality of Black Soldier Fly (BSF)-derived products.

Water hyacinth *(Eichhornia crassipes*), an invasive aquatic plant found on many water bodies, has been both a nuisance and a menace to society for years. Punitha et al. (2024) outlined several beneficial uses of the plant, including its applications in phytoremediation, water purification and biodiesel production. However, its excessive proliferation in aquatic environments is still highly problematic and often outweighs the potential benefits. Water hyacinth contributes to declining fish stocks, obstructs fishing activities, and endangers aquatic life by reducing dissolved oxygen levels and degrading overall water quality (Asmare, 2017; Hussien, 2020;Lubembe et al., 2023). Despite its negative ecological impacts, water hyacinth represents a readily available biomass resource with great potential for valorisation. Moreover, Belete et al. (2022) have identified that water hyacinth possesses considerable concentrations of crude protein, crude fibre and carbohydrates in its leaves. They found approximately 18.03%, 31.04% and 22.30% crude protein, crude fibre and carbohydrate concentrations, respectively, in the leaves of water hyacinth. The moderate nutrient levels, high moisture content and annual productivity of the plant make it a candidate for use as a component of BSFL rearing substrates. Nevertheless, the high fibre content of the plant and its relatively low available protein may limit larval performance if used alone. Hence, blending water hyacinth with nutrient-rich substrates, such as para rubber seed, fruit waste and pig manure, may improve substrate quality and promote larval growth. Beyond water hyacinth, the organic waste streams that were selected for blending in this study -para rubber seed, fruit waste and pig manure- are substrates with considerable potential for BSFL growth. Para rubber seeds contain approximately 26.1% crude protein (Hossain et al. 2015). Despite this advantageous nutrient profile, they remain an underutilised protein-rich agricultural by-product, with limited application in BSF rearing. This could be due to the presence of anti-nutritional factors (ANFs) including oxalate, tannin and phytate in para rubber seeds. However, a significant reduction in ANFs was observed when para rubber seeds were boiled or fermented (Agbai et al. 2021). Furthermore, fruit waste and pig manure have been extensively used in BSF studies due to their availability and favourable nutrient compositions.

Notwithstanding their relevance, empirical studies exploring combined substrate formulations comprising these wastes and water hyacinth remain scarce. Understanding how water hyacinth-based substrates influence BSFL growth and life history traits is crucial for optimising bioconversion efficiency and enabling the sustainable use of this invasive plant in insect-based feed production systems. Despite existing research on water hyacinth utilisation in livestock feed production in Ghana, information on its influence on insect-based systems is lacking, with no published studies focusing on BSFL. Therefore, the overall aim of this study was to evaluate the growth, developmental performance, and reproductive outcomes of BSFL reared on water-hyacinth-formulated substrates, to determine their suitability for sustainable insect biomass production. The study assessed larval growth performance, prepupation success, adult emergence and egg viability across various substrate blends. Multivariate trends in larval development across substrates and time were examined using Non-metric Multidimensional Scaling (NMDS).

Material and methods

Study area

The study was conducted at a Black Soldier Fly rearing facility situated within the Department of Theoretical and Applied Biology (TAB) at KNUST (6.67315^{0}N, -1.56915^{0}W), in the Ashanti Region of Ghana. The facility consisted of a Larvarium, where larvae were fed and developed, and an Insectarium, where adult flies lived and bred. To promote mating activities, clear fibreglass roofing was used in the fly house to increase light intensity and warmth. The lower section of the structure was constructed from concrete, while the upper section was enclosed with mesh screening to enhance ventilation, prevent adult fly escapes and keep predators out. The experiment was undertaken from October 2024 to December 2024.

Preparation of experimental substrates

The experimental substrates consisted of four (4) organic waste streams (Water hyacinth, Para rubber seed, Fruit waste and Pig manure), combined in various proportions. Water hyacinth was harvested from the Volta Lake in the Volta Region of Ghana. The leaves, stems and roots of the plant were collectively milled into pulp using a hammer mill. Para rubber seeds were collected from a commercial rubber plantation at Asiakwa, Eastern Region. Following the method of Matho et al. (2021), the seeds were decorticated and cooked in boiling water at 100°C for 45 minutes. Afterwards, the seeds were sun-dried and milled. This was done to reduce anti-nutritional factors (ANFs) in the resulting substrate. Fruit waste (comprising peels, pulp and seeds of pineapple, pawpaw and watermelon) was collected with jute sacks from the Abinkyi Market in Kumasi. Fruit waste was chopped into pieces (~ 2cm) and combined in a 1:1:1 ratio before use. A day before the trial, pig manure was obtained from the Animal Science Department, KNUST. They were stored in sealed plastic containers at 4°C and homogenised thoroughly before allocation into experimental units.

Figure 1. *A graphical illustration of experimental substrates. The control diet used was chicken feed*

Bioassays

Black soldier fly larvae

Black soldier fly larvae used in the study were obtained from a previously established colony at the BSF facility at TAB (KNUST). The larvae were reared on moistened chicken feed (1:1 v/v) in the Larvarium (29°C ± 4, 67.5% ± 10.61 relative humidity (RH), and 12L:12D photoperiod) until they transitioned into prepupae. Prepupae were transferred to the Insectarium (28°C ± 4°C, 48% ± 5% RH, and 12L:12D photoperiod), where the adults eclosed.

Oviposition devices, each made from two slats of wood secured with rubber bands at both ends, were placed on ventilated containers carrying fermented chicken feed in the Insectarium. Eggs laid within 24 hours were carefully removed from the oviposition devices using a sharp scalpel and then placed on a dry petri dish. Following the procedure of Nyakeri et al. (2017), the petri dish was placed inside a container (21cm x 15cm x 4cm) containing chicken feed that had been well-moistened with water to a moisture content of 60%. A hole was created on the lid of the container, and the cut area was replaced with muslin

cloth to allow for aeration and avoid larval escape. The container was kept under the same environmental conditions described above.

Morphometric traits of BSFL

A total of 300 hand-counted 5-day-old larvae were transferred onto 90g (100mg/larva/day) of each experimental diet in a 1L container, following the procedures of Diener et al. (2009). The containers were covered with muslin cloth and secured with jute strings. The experiment was set up in quadruplicates, arranged in a completely randomised design and lasted for 15 days. A continuous feeding approach was employed, where larvae were fed every 3 days. Every 3rd day, all larvae were separated from the feed. Live and dead larvae were counted and recorded, returning only the live larvae to the supplied fresh feed. Each setup was also monitored daily for the transition of larvae into prepupae. Ten (10) larvae were randomly sampled and dabbed with a tissue to remove feed particles. The collective weight of the 10 larvae was obtained using a digital weighing scale with a precision of 0.001g (Ohaus, PR series). Their individual lengths were measured with a ruler, and their head capsule measurements were obtained with a digital calliper (Paget Services, version 06/2007; precision = 0.001mm). After measurements were taken, they were returned to their respective containers. The residual substrates were stored in ziplock bags at -20^{0}C °C until further analysis. When 50% of larvae on any substrate transitioned into prepupae, feeding was stopped, and the experimental unit was censored from the trial (Sheppard et al. 2002).

Oviposition and egg hatchability

When adults emerged inside the wooden cages, balls of cotton wool were soaked in water and placed in small plastic cups inside the cages to maintain humidity and sustain the adult flies. Oviposition materials were made by securing 2 rectangular slats of wood (15 cm x 6 cm) placed side-by-side with rubber bands. In the cages, the stacked slats were placed on top of containers housing fermented chicken feed, which served as an oviposition attractant medium. Egg clutches were collected once from oviposition traps following standard procedures for BSF reproductive assessment (Sheppard et al. 2002). Unaffected eggs were collected from the crevices between the stacked wood and weighed. Three (3) samples of 100 eggs per treatment were used for the egg hatchability assessment (Maïga et al. 2020). Adapting the procedures of Hoc et al. (2019), two independent sets of 100 eggs per clutch were counted, and a fine camel hair brush was used to spread them evenly on pieces of 7cm x 5 cm black eggshell paper, inside 9mm petri dishes. The eggshell paper allowed easy inspection of the eggs. The petri dishes were then placed inside 21cm x 15cm x 4cm containers and covered until hatching. In the first set, unhatched eggs were counted using a stereomicroscope, two days after the eggs hatched. Percentage hatchability was estimated using the formula below;

Percentage hatchability = $\frac{number\ of\ hatched\ eggs}{total\ number\ of\ eeggs}\ x\ 100$ .....Eq. 1

The second set of petri dishes was placed atop 80g of moistened cshick mash. The setups were maintained at 25–32°C and 63–77% RH (Sheppard et al. 2002). On the 5th day, a subsample of ten (10)

larvae was randomly selected per replicate, and their mean weights were obtained to estimate early larval biomass.

Adult eclosion and sex ratio

Two independent sets of 100 prepupae were obtained from each setup. One set was used for assessing adult emergence, while the other was used to determine the adult sex ratio. They were placed on wood shavings in 200 ml disposable cups and kept in separate netted wooden cages. Newly emerged adults were counted and recorded daily. Adults were sexed 24–48 hours post-eclosion, using established morphological markers (Cai et al., 2022).

After sexing, ten (10) adult flies (5 males and 5 females) were transferred into a ventilated container. The container was then placed on ice for approximately 2 minutes to immobilise the flies, after which their individual weights were quickly obtained using a sensitive digital weighing scale (Ohaus, PR series; 0.001g precision). Flies were returned to their respective containers after weighing and monitored until larvae had fully recovered.

Nutrient profile analysis

The proximate composition of the rearing substrates (crude protein, fat, crude fibre, ash, nitrogen-free extract (NFE) and moisture content) was determined using standard analytical methods based on the procedures outlined by the Association of Official Analytical Chemists (AOAC 2000). All analyses were performed in triplicate to ensure reliability, and results were expressed on a dry matter basis.

Data analysis

All statistical analyses were performed using the R statistical software package (version 4.4.3). Levene and Shapiro-Wilk tests were used to check for normality and homogeneity of variances, where ANOVA was performed. Tukey's Honest Significant Difference was used to separate means, where significant differences were obtained. The overall effects of treatment on response variables were assessed using a PERMANOVA test, as implemented in the adonis2 package in R. The level of significance for all analyses was set at $p < 0.05$.

Results

Impact of feeding substrate and time on BSFL growth traits

Significant differences were observed in the proximate composition across the eleven feeding substrates (Table 1). Fat content ranged from 0.92% in SMWH-C (20:80 water hyacinth to pig manure) to 17.69% in PRWH-C (20:80 water hyacinth to milled para rubber seed), with the three distinct milled para rubber seed and water hyacinth substrate blends (PRWH) generally exhibiting the largest fat levels compared to the fruit waste and water hyacinth blends (FWWH). Fibre content also varied considerably, with FWWH-A (50:50 fruit waste to water hyacinth) containing the highest proportion (42.56%), while 100% chicken feed (CF) recorded

the least (8.12%). Ash content showed substantial variation, ranging from 1.41% in FWWH-A to 25.12% in SMWH-C. Moisture content ranged from 23.62% in CF to 42.42% in FWWH-A, with FWWH consistently exhibiting the highest moisture levels. The lowest protein levels were recorded in FWWH-C (7.29%) and SMWH-C (7.35%), whereas PRWH-C recorded the largest protein concentration (28.26%) (Table 1). Carbohydrate (%), calculated by difference, ranged from 1.18% in the composite mixture of all four organic substrates (WPSF) to 31.25% in CF.

Table 1
Proximate composition (%) of the feeding substrates used for BSFL rearing

FEEDING SUBSTRATE	FAT	FIBER	ASH	MOISTURE	PROTEIN	CARBOHYDRATE
CF	5.89 ± 0.01 e	8.12 ± 0.01 a	7.05 ± 0.02 f	23.62 ± 0.00 a	24.08 ± 0.25 g	31.25 ± 0.25 g
FWWH(A)	1.95 ± 0.01 c	42.56 ± 0.05 k	1.41 ± 0.12 a	42.42 ± 0.00 i	9.28 ± 0.05 b	2.39 ± 0.17 cd
FWWH(B)	2.12 ± 0.04 c	38.54 ± 0.07 i	3.41 ± 0.02 b	42.30 ± 0.02 i	11.03 ± 0.05 d	2.60 ± 0.11 cd
FWWH(C)	1.96 ± 0.01 c	40.36 ± 0.09 j	3.17 ± 0.06 b	41.50 ± 0.02 h	7.29 ± 0.08 a	5.71 ± 0.15 e
PRWH(A)	12.61 ± 0.04 g	25.24 ± 0.06 e	4.88 ± 0.04 c	30.51 ± 0.01 d	24.78 ± 0.05 h	1.98 ± 0.11 bc
PRWH(B)	6.90 ± 0.04 f	24.20 ± 0.03 d	5.71 ± 0.02 d	35.99 ± 0.00 f	25.89 ± 0.08 i	1.31 ± 0.09 ab
PRWH(C)	17.69 ± 0.09 i	11.27 ± 0.13 b	6.12 ± 0.01 e	27.83 ± 0.21 b	28.26 ± 0.10 j	8.84 ± 0.20 f
SMWH(A)	2.70 ± 0.06 d	23.53 ± 0.07 c	24.12 ± 0.01 i	34.04 ± 0.00 e	10.07 ± 0.05 c	5.54 ± 0.11 e
SMWH(B)	1.56 ± 0.09 b	30.55 ± 0.05 g	16.44 ± 0.10 h	37.85 ± 0.00 g	11.68 ± 0.08 e	1.91 ± 0.18 abc
SMWH(C)	0.92 ± 0.05 a	35.20 ± 0.03 h	25.12 ± 0.01 j	28.33 ± 0.00 c	7.35 ± 0.10 a	3.07 ± 0.13 d
WPSF	14.67 ± 0.09 h	27.17 ± 0.06 f	10.21 ± 0.02 g	30.74 ± 0.00 d	16.02 ± 0.05 f	1.18 ± 0.03 a

Values represent mean ± standard error (SE). Different superscript letters within the mean hatchability percentage column indicate significant differences among substrate treatments based on one-way ANOVA followed by Tukey's HSD post-hoc tests at p < 0 .05. All other parameters showed no statistically significant differences across treatments. WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to

water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

A Permutational Multivariate Analysis of Variance (PERMANOVA) was used to evaluate the main and interaction effects of time and treatment on larval weight, length, and head capsule width. The results showed highly statistically significant main effects for both feeding substrate ($F_{10,193} = 70.33$, $p < 0.001$) and time ($F_{5,193} = 63.76$, $p < 0.001$). This indicates that the different substrate compositions and different periods of exposure to substrates independently had significant impacts on the overall structure of the response variables. Furthermore, the interaction effect between feeding substrate and time was significant ($F_{50,193} = 8.23$, $p < 0.001$), suggesting that treatment effects on response variables varied depending on the day, and likewise, changes over time varied across treatments. A substantial proportion of the total variance was explained by the Treatment factor ($R^2 = 0.432$).

Figure 1 is a visual representation of the PERMANOVA-based pairwise comparison results for BSFL mean weight between treatment groups. No statistically significant differences were found across the five best-performing host diets (PRWH, CF and WPSF) comparisons ($p > 0.05$). Likewise, no significant differences existed between the pig manure and water hyacinth blends (SMWH) and FWWH-B substrates ($p > 0.05$). There were some differences within the FWWH, specifically between FWWH-A and FWWH-B ($p = 0.015$). Additionally, FWWH-B was significantly different from FWWH-C ($p = 0.001$). Overall, most treatment group comparisons were found to be statistically significant ($p < 0.05$). Notably, comparisons between PRWH and FWWH, as well as PRWH and SMWH, revealed highly statistically significant differences ($p = 0.000$). A similar outcome was observed in both WPSF and CF when compared with FWWH and SMWH, respectively ($p = 0.000$).

Notes. Cells display adjusted p-values (Benjamini–Hochberg correction) with significance codes ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***; ns = not significant). PERMANOVA tests were performed using 999 permutations. Darker blue = stronger significance (Adj. $p < 0.001$); red/orange = significant at Adj. $p < 0.05$; white/light blue = nonsignificant (ns). WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

The pairwise PERMANOVA results for BSFL mean length are visually represented in Fig. 2. No significant differences were observed between CF and either WPSF or PRWH substrates (Fig. 3). Similarly, WPSF was not significantly different from PRWH substrates ($p > 0.05$). In contrast, significant differences existed between CF and both FWWH and SMWH substrates. Additionally, when WPSF and PRWH substrates were individually contrasted with FWWH and SMWH in pairwise comparisons, highly significant differences were observed ($p < 0.05$). Significant differences were generally observed between FWWH and SMWH ($p < 0.05$).

However, no significant differences were detected in comparisons involving FWWH-B and either SMWH-C ($p = 0.696$) or SMWH-A ($p = 0.089$). Internal variations were evident within both FWWH and SMWH substrates. For instance, a high statistically significant difference emerged from the comparison of FWWH-B and FWWH-C ($p = 0.007$), as well as between SMWH-B and SMWH-C ($p = 0.01$).

Notes. sCells display adjusted p-values (Benjamini–Hochberg correction) with significance codes ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***; ns = not significant). PERMANOVA tests were performed using 999 permutations. Darker blue = stronger significance (Adj. $p < 0.001$); red/orange = significant at Adj. $p < 0.05$; white/light blue = nonsignificant (ns). WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

Figure 3 displays the pairwise PERMANOVA comparison results for the mean head capsule of BSFL across treatment groups. Similar to Figs. 1 and 2, no significant differences were found between PRWH, CF and WPSF substrates. However, highly statistically significant differences emerged between them and either FWWH or SMWH substrates. Significant differences existed between SMWH and FWWH-C, as well as between FWWH and SMWH-B ($p < 0.005$).

Notes. Cells display adjusted p-values (Benjamini–Hochberg correction) with significance codes ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***; ns = not significant). PERMANOVA tests were performed using 999 permutations. Darker blue = stronger significance (Adj. $p < 0.001$); red/orange = significant at Adj. $p < 0.05$; white/light blue = nonsignificant (ns). WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

Figure 4 displays the interaction effects of trearing substrates and day on the mean weight of BSFL. Except for SMWH-A and SMWH-B, the other treatments showed a steady increase from Day 0 until day 15. It can be observed from the plot that WPSF, PRWH and CF performed comparatively better than FWWH and SMWH throughout the entire duration of the study. Mean weight values ranging from 157.08 mg to 194.69 mg were recorded for larvae reared on these substrates by day 12. On day 12, WPSF was terminated as 50% of the larvae had transitioned into the prepupal stage. On day 15, PRWH-A exhibited the largest mean weight (210.46 mg), while the least was recorded on SMWH-B (14.97 mg).

Notes. Estimated marginal means of BSFL weight (mg) over time. Estimated marginal means (± SE) of larval weight across days and feeding substrates derived from a robust linear model. Pairwise comparisons were performed using the emmeans package with Tukey adjustment at $p = 0.05$. Higher trajectories indicate faster morphological development across feeding substrates. WPSF = water hyacinth, milled para rubber

seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

Figure 5 depicts the interaction effects of tsubstrates and day on the mean length of BSFL. The patterns portrayed in Fig. 5 mirror the trends observed in Fig. 4. The para rubber-hyacinth (PRWH) substrates, WPSF, and CF outperformed the FWWH and SMWH substrates. At the end of the study period, PRWH-A yielded the longest BSFL (21.65 mm), whereas SMWH-B produced the shortest (8.1 mm).

Estimated marginal means of BSFL length over time. Estimated marginal means (± SE) of larval length across days and feeding substrates derived from a robust linear model. Pairwise comparisons were performed using the emmeans package with Tukey adjustment at p = 0.05. Higher trajectories indicate faster morphological development across feeding substrates. WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

The interaction effects of treatment and day on the mean head capsule width of BSFL have been presented in Fig. 6. As shown in the figure, the interaction of substrates and day significantly impacted the head capsule widths of BSFL. The longest head capsule widths were found in larvae reared on PRWH, WPSF and CF. Mean head capsule widths of BSFL ranged from 0.01 mm (SMWH-B) to 0.21 mm (PRWH-A) on day 15.

Pairwise comparisons were performed using the emmeans package with Tukey adjustment at p = 0.05. Higher trajectories indicate faster morphological development across feeding substrates. WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

Figure 7 illustrates the results of a Non-metric Multidimensional Scaling (NMDS) ordination, which was performed to visualize the similarity and dissimilarity of BSFL growth trait profiles across different treatments and data collection time points. The NMDS ordination achieved a very low stress value of 0.009, which indicates a better fit of the ordination to the data. The NMDS ordination in Fig. 7 clearly demonstrates that both Treatment and Day significantly influenced the overall growth characteristics of BSFL. The NMDS revealed clear temporal effects and moderate treatment variation in the multivariate response of BSF larvae across substrates. Early-stage samples (Day 0–3) clustered tightly together, indicating similar initial larval profiles regardless of substrate. As development progressed, later sampling days (particularly Days 9–15) became more dispersed in ordination space, reflecting significant growth and variation in larval traits over time. Subtle substrate-specific groupings also emerged, with some treatments (PRWH-B, SMWH-B, and

WPSF) forming more distinct ellipses than others, suggesting moderate but detectable treatment-level effects on overall larval development trajectories. The different treatment groups (represented by the ellipses in the figure) significantly impacted BSFL growth trait profiles. This is indicated by the varying degrees of clustering and separation of the ellipses. During the later stages, SMWH substrate formed a distinct cluster at the lower end of the NMDS2 axis, indicating lower growth performance. In the central and upper right regions of the plot, extensive overlapping was detected between PRWH, WPSF and CF, suggesting that they share similar growth patterns (high growth performance).

Ellipses represent 95% confidence regions around treatment centroids. Analysis was performed using PERMANOVA (999 permutations) with a significance threshold of p = 0.05. WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWHC = 80:20 pig manure to water hyacinth, SMWHB = 20:80 pig manure to water hyacinth, SMWHA = 50:50 pig manure to water hyacinth, PRWHC = 80:20 milled para rubber seed to water hyacinth, PRWHB = 20:80 milled para rubber seed to water hyacinth, PRWHA = 50:50 milled para rubber seed to water hyacinth, FWWHC = 80:20 fruit waste to water hyacinth, FWWHB = 20:80 fruit waste to water hyacinth, FWWHA = 50:50 fruit waste to water hyacinth, CF = chicken feed

Substrate effects on developmental trajectories and median development time (D_{50}) of BSFL

By day 6, peak probabilities above 0.90 were reached by instar 4 in the milled para rubber seed and water hyacinth substrates (PRWH) and the WPSF substrate. Conversely, delayed peaks were observed for both the FWWH and SMWH substrates from Day 12–15. The highest probability of Instar 6 (0.80) was observed on WPSF by day 15. This was followed by PRWH-A (0.38), whereas SMWH and FWWH substrates showed negligible progression to Instar 6 (~ 0.00).

Panels show predicted probabilities for instars 2–6 across sampling days for each substrate; multinomial logistic regression (glm) with substrate and day as predictors; pairwise comparisons adjusted using the Tukey method (p = 0.05). WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH–C = 80:20 pig manure to water hyacinth, SMWH–B = 20:80 pig manure to water hyacinth, SMWH–A = 50:50 pig manure to water hyacinth, PRWH–C = 80:20 milled para rubber seed to water hyacinth, PRWH–B = 20:80 milled para rubber seed to water hyacinth, PRWH–A = 50:50 milled para rubber seed to water hyacinth, FWWH–C = 80:20 fruit waste to water hyacinth, FWWH–B = 20:80 fruit waste to water hyacinth, FWWH–A = 50:50 fruit waste to water hyacinth, CF = chicken feed

Figure 9 presents the substrates that reached 50% pre-pupation in the course of the study. The bar plots, generated from the observed timepoints, revealed distinct patterns in pre-pupation success among the treatments.

At day 12, the WPSF treatment exhibited the earliest significant result, with all four replicates (100%) reaching 50% pre-pupation. The CF also showed activity at day 12, with one replicate reaching 50% pre-pupation, followed by all four replicates (100%) by day 15, reflecting a delayed but complete progression. In contrast, PRWH-A and PRWH-B demonstrated a uniform increase, with all four replicates per treatment reaching 50% pre-pupation by day 15, indicating a consistent but delayed response compared to WPSF and CF.

Water hyacinth and para rubber seed meal in a 20:80 ratio (PRWH-C) showed a mixed response, with only one of four substrates (25%) reaching 50% pre-pupation by day 15, indicating limited effectiveness. The FWWH and SMWH substrates exhibited no incidence of 50% pre-pupation, with all replicates censored at day 15, resulting in no bar plots.

Proportion of substrates reaching 50% prepupation (left) and days to 50% prepupation for successful replicates only (right) across feeding substrates. Bars show the number of successful replicates out of four per treatment. Differences among substrates were evaluated using one–way ANOVA (p = 0.05) for the number of days to 50% prepupation; treatments failing to reach 50% prepupation were excluded from ANOVA and plotted descriptively only. WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH–C = 80:20 pig manure to water hyacinth, SMWH–B = 20:80 pig manure to water hyacinth, SMWH–A = 50:50 pig manure to water hyacinth, PRWH–C = 80:20 milled para rubber seed to water hyacinth, PRWH–B = 20:80 milled para rubber seed to water hyacinth, PRWH–A = 50:50 milled para rubber seed to water hyacinth, FWWH–C = 80:20 fruit waste to water hyacinth, FWWH–B = 20:80 fruit waste to water hyacinth, FWWH–A = 50:50 fruit waste to water hyacinth, CF = chicken feed

Development time and larval growth performance

From Table 2, the shortest larval development time (9 days) was observed on the control feed (CF), PRWH-A and WPSF. PRWH-A recorded the largest biomass gain and average growth rate (20.66g and 1.38g, respectively), while SMWH-B exhibited the least (1.66g and 0.11g, respectively). In relation to Mean Biomass Gain and Average Growth Rate, there were significant differences between PRWH-A and the other substrates, except CF, PRWH-B, PRWH-C and WPSF. It was significantly different from all the other substrates, including CF. However, PRWH-B, PRWH-C and WPSF did not differ significantly from CF. The least biomass gain was recorded on SMWH-B while PRWH-A showed the highest average growth rate.

Table 2
Summary of development time (DT), biomass gain (BG), and absolute growth rate (AGR) of BSFL reared on eleven substrates, including means ± SD ($p \leq 0.05$).

Feeding Substrate	Mean DT ± SD (days)	Mean BG ± SD (g DW)	Mean AGR ± SD (g DW/day)
CF	9.00 ± 0.00 [a]	12.93 ± 3.24 [a]	0.910 ± 0.203 [a]
FWWH(A)	15.00 ± 0.00 [b]	5.18 ± 1.89 [b]	0.350 ± 0.126 [b]
FWWH(B)	15.00 ± 0.00 [b]	2.3 ± 0.23 [b]	0.150 ± 0.015 [b]
FWWH(C)	15.00 ± 0.00 [b]	5.52 ± 1.17 [b]	0.370 ± 0.078 [b]
PRWH(A)	9.00 ± 0.00 [a]	20.66 ± 5.50 [c]	1.380 ± 0.367 [c]
PRWH(B)	9.75 ± 1.50 [ac]	14.39 ± 2.75 [a]	0.960 ± 0.183 [a]
PRWH(C)	11.25 ± 1.50 [c]	17.49 ± 2.49 [ac]	1.170 ± 0.166 [ac]
SMWH(A)	15.00 ± 0.00 [b]	2.14 ± 0.85 [b]	0.140 ± 0.057 [b]
SMWH(B)	15.00 ± 0.00 [b]	1.66 ± 0.56 [b]	0.110 ± 0.038 [b]
SMWH(C)	15.00 ± 0.00 [b]	2.59 ± 0.86 [b]	0.170 ± 0.057 [b]
WPSF	9.00 ± 0.00 [a]	15.61 ± 2.19 [ac]	1.30 ± 0.182 [ac]

Development Time (DT) measured from placement of 5–day–old larvae to first prepupa, monitored every 3 days (days 0, 3, 6, 9, 12, 15). Biomass gain (BG) = final biomass – initial biomass. Average Growth Rate (AGR) = biomass gain / 15 days, except for WPSF and CF, where some replicate(s) use 12 days. CLD letters indicate significant differences (Tukey's HSD, $p < 0.05$); treatments sharing the same letter are not significantly different. WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

Influence of substrate type on adult outcomes, egg metrics and larval biomass

No significant differences were found in adult sex ratio (proportion males) and sex ratio across the five best-performing substrates that reached the prepupal stage. Similarly, there were no significant differences across substrates in terms of mean weights of male and female BSF, as well as adult emergence counts (Table 3). However, percentage hatchability showed significant differences across rearing substrates. The control (CF) recorded the largest number of hatched eggs (97.75%). No significant differences were found in WPSF in relation to CF, whereas PRWH-A and PRWH-C were significantly different from CF. The lowest percentage of hatchable eggs was recorded on PRWH-B (15%). Although the number of eggs that hatched

varied across substrates, the mean weights of ten 5-day-old larvae that were randomly sampled from each substrate showed no significant differences (Table 3).

Table 3
Mean ± SD of Adult Developmental and Reproductive Performance of Black Soldier Fly Across Substrate Treatments

Feeding Substrate	Mean egg biomass (g)	Mean hatchability percentage (%)	Mean larval biomass (g)	Mean male adult count	Mean female adult count	Mean male adult weight (g)	Mean female adult weight (g)	Mean sex ratio (proportion male)
CF	0.018 ± 0.009	97.75 ± 2.88 ^{a}	0.416 ± 0.028	39.00 ± 15.01	40.75 ± 7.80	0.048 ± 0.011	0.056 ± 0.003	0.520 ± 0.116
PRWH(A)	0.011 ± 0.010	51.25 ± 15.78 ^{bc}	0.292 ± 0.238	35.75 ± 1.26	30.75 ± 7.27	0.054 ± 0.004	0.062 ± 0.006	0.458 ± 0.057
PRWH(B)	0.012 ± 0.012	15.00 ± 0.82 ^{c}	0.004 ± 0.000	30.00 ± 5.89	35.50 ± 7.72	0.054 ± 0.007	0.063 ± 0.008	0.540 ± 0.089
PRWH(C)	0.008 ± 0.003	30.00 ± 16.84^{bc}	0.004 ± 0.002	31.00 ± 3.74	31.75 ± 2.06	0.057 ± 0.006	0.058 ± 0.008	0.507 ± 0.042
WPSF	0.019 ± 0.014	60.25 ± 36.34 ^{ab}	0.585 ± 0.632	38.75 ± 9.78	31.00 ± 4.76	0.056 ± 0.002	0.061 ± 0.009	0.449 ± 0.080

Values represent mean ± standard error (SE). Different superscript letters within the mean hatchability percentage column indicate significant differences among substrate treatments based on one–way ANOVA followed by Tukey's HSD post–hoc tests at p < 0 .05. All other parameters showed no statistically significant differences across treatments. CF = chicken feed, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH (C) = 80:20 milled para rubber seed to water hyacinth, WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio,

DISCUSSION

Influence of Substrate and Time on BSFL Growth Traits

This study was conducted to determine how various combinations of organic waste streams (water hyacinth, milled para rubber seed, fruit waste and pig manure) influenced the growth and reproductive performance of black soldier fly throughout a 15-day observation period. The substrate composition significantly influenced the growth performance of BSFL, as evidenced by the highly significant effects of feeding substrates. The effects of period of exposure and its interaction with the various feeding substrates on growth performance metrics (weight, length and head capsule width of larvae) were highly significant. Weight, length and head capsule width of larvae increased steadily throughout the study period. Compared to fruit waste / water hyacinth (FWWH) and pig manure / water hyacinth (SMWH) substrates, larvae reared

on para rubber / water hyacinth (PRWH) substrate, composite substrate composed of all four organic wastes (WPSF) and chicken feed (CF) showed superior growth metrics. The trend observed for the interaction effects of feeding substrates and time on larval weight mirrors the results reported by Yakti et al. (2023). According to Albalawneh et al. (2024), substrate composition plays a key role in enhancing BSFL biomass production. The remarkable growth trait dynamics observed in larvae corroborate the strong relationship between substrate nutritional quality and larval growth performance.

There were no significant differences across the five (5) best-performing substrates (three PRWH variants, WPSF and CF). Similar results were observed by Mulu et al. (2023), who examined the effects of varying proportions of water hyacinth, fruit waste, and manure on BSFL. Their study revealed that BSFL cultivated on the aforementioned substrates in a ratio of 35:55:10 demonstrated superior growth performance over the study period. The most effective feeding substrate in this study (PRWH-A), comprising an equal proportion of water hyacinth and milled para rubber seed, supported the fastest development time and highest growth performance in BSFL. This observation may be attributed to the high concentrations of protein and fat in PRWH-A. Additionally, the balanced formulation likely provided an optimal blend of nutrients, particularly protein and fat, essential for larval development. A positive correlation exists between protein and larval biomass (Dortmans et al. 2021; Hosseindoust et al., 2023). This relationship was further demonstrated by Somarny et al. (2023), who reported that protein-dense substrates (Sesbania grandiflora, Moringa oleifera leaves and Soybean waste) produced the highest larval biomasses. Regarding the effects of fat on larval growth, mixed results have been presented in the literature. For instance, while the findings ofLi et al. (2022) indicate that fat-rich substrates enhance growth in BSFL,Knudsen et al. (2022) noted that high lipid levels in BSFL substrates correlated with poor growth performance in larvae. The mixed results may be attributed to the lipid composition of and probable influence of other components of the substrates, such as anti-nutritional compounds. Optimal larval performance observed in both PRWH-B (80:20 water hyacinth to milled para rubber seed) and PRWH-C (20:80 water hyacinth to milled para rubber seed) may be a result of the similar nutrient profile they share with PRWH-A. Feeding substrates which contained higher proportions of water hyacinth, such as FWWH-B (80:20 water hyacinth to fruit waste) and SMWH-B (80:20 water hyacinth to pig manure), were associated with significantly reduced larval growth performance. Despite PRWH-B also being a water hyacinth-dominant substrate, it demonstrated a reverse pattern. This could have been due to the high protein content of PRWH-B. The findings obtained for FWWH-B and SMWH-B align with previous observations by Mulu et al. (2023), who reported poor outcomes in substrates dominated by water hyacinth. Wahyuni and Fadhlil (2022) reported comparable findings, noting that water hyacinth–dominated substrates exhibit poor performance. Their study achieved optimal larval growth performance with a 75:25 ratio of fruit waste to water hyacinth. Similarly, in the present study, FWWH-A (50:50 water hyacinth to fruit waste) outperformed the other substrates in the FWWH group. However, compared to PRWH substrates, BSFL growth performance on FWWH substrates was consistently lower across all measured growth parameters. The poor growth performance of BSFL in water hyacinth-dominated substrates may be attributed to the relatively low nutritional value of water hyacinth (Jimoh et al. 2016). This highlights the importance of balancing substrates with nutrient-rich co-substrates in BSFL feed formulations to enhance growth performance.

Wamboga et al. (2025) recorded the highest weight gain and length of BSFL on mixed substrates. Likewise, larvae reared on WPSF attained remarkable growth performance metrices. The combination of the four organic wastes may have yielded a more nutrient-dense substrate for larval growth. According to Weko et al. (2023), the drivers of weight gain in BSFL are digestibility, nutrient composition and palatability of feeding substrates. In their study,Albalawneh et al. (2024) found that 100% chicken feed produced larvae with the greatest body size among all the experimental diets. This concurs with the findings of this study, where commercial chicken feed (control feed) emerged as one of the best-performing substrates. Commercial chicken feed performed exceptionally well due to its high digestibility and balanced macro and micronutrient composition. The production of the shortest and lightest larvae on SMWH substrates aligns with the findings of Hudson et al. (2019), who observed a similar trend when larvae reared on swine droppings demonstrated the worst performance in terms of morphometric traits. The ash content in SMWH substrates was highest across the feeding substrates.Hao et al. (2023) observed that an increase in the ash content of BSFL substrates accounted for a decrease in developmental performance of larvae and reduced key larval metrics like larval weight and larval length. Therefore, the poor growth performance exhibited by larvae reared on SMWH substrates may be attributed to the high quantities of ash present in these substrates. In addition, a study conducted byWeko et al. (2023) revealed that BSFL that were harvested on day 15 contained high levels of ash compared to larvae that were harvested on day 20. In light of this, the rearing duration of this study may have exacerbated the already-high ash content outcomes of SMWH-reared larvae, contributing to their poor developmental performance. Pig manure and water hyacinth blends (SMWH) were characterized by limited protein, low-fat content and a fibre-rich composition, followed by FWWH substrates. The reduced growth performance in the SMWH-reared larvae may be attributed to low concentrations of protein and fat in the substrate.Laganaro et al. (2021) found that as the proportion of degassed sludge (a low-nutrient substrate) increased, larvae used more assimilated feed for maintenance metabolism and spent more energy on respiratory processes instead of growth. Furthermore, the presence of high levels of fibre in substrates is known to be associated with poor performance in larvae (Liu et al. 2018; Yakti et al. 2023). The differences observed in the interactions across substrates and period of exposure may be attributed to variations in substrate composition, nutrient availability and feed digestibility. The five (5) best-performing substrates were composed of high contents of protein and fat, two key components essential for optimal development of BSFL. In contrast, the poor-performinge substrates (FWWH and SMWH) contained high levels of fibre and ash, which are known to impede larval growth (Grossule et al. 2020; Hosseindoust et al. 2024). The findings ofNaser El Deen et al. (2023) confirm this by recording the lowest efficiency of conversion in larvae reared on pig manure slurry mixed with silage grass.

Substrate effects on larval developmental performance and prepupation dynamics

The shortest development time recorded on PRWH-A and WPSF may be due to the relatively high protein content of these substrates. Similarly,Somarny et al. (2023) observed that Soybean waste (a high-protein substrate) supported the fastest larval development.Reis (2016) reported that high-protein diets supported a rapid development in *Drosophila* sp. Compared to PRWH-A, larvae reared on PRWH-B exhibited slower development. In contrast, high-fat diets were found to have a negative impact on development. This may be the reason for the relatively longer larval development time that was observed in PRWH-C, as this substrate

contained the highest level of fat. Additionally, the relatively longer larval development time recorded in larvae reared on PRWH-C may be attributed to nutritional imbalances. Although the overall highest fat and protein contents were observed in PRWH-C, the moisture content of this substrate was low. This substrate was also found to have a low fibre content. Substrates with low fibre and suboptimal moisture levels have been found to prolong development time in BSFL (Opare et al. 2024;Cammack and Tomberlin 2017). Notably,Bekker et al. (2021) observed that substrates with moisture contents outside the range of 45–75% tend to extend the development time of larvae.Reis (2016) discovered that high-carbohydrate diets prolonged development in *Drosophila* sp. Conversely, the high carbohydrate content of CF did not delay larval development in this study; instead, it was among the substrates that promoted rapid larval growth.

The slow larval development on both FWWH and SMWH substrates is consistent with the findings of Lalander et al. (2019), who observed slower development on substrates composed of fruits and vegetables. This may be due to the relatively low protein and fat composition of these substrates. According toMukarugwiro et al. (2018), nutrient concentration is a strong predictor of development time. Protein and fat are crucial nutrients for larval yield, yet these substrates were found to contain extremely low levels of these two essential nutrients.

Among the 5 best-performing substrates, WPSF was the first to reach the 50% prepupation milestone. In their study, Shamini and Fauziah (2014) noted an improvement of about 64% in waste degradation when multiple substrates were combined in equal proportions. Mulu et al. (2023) found that substrates that had high reduction efficiencies promoted development in larvae, after they reared BSFL on a formulated diet of water hyacinth, fruit waste and manure in a ratio of 35:55:10. Moreover, Nemet et al. (2021) indicated that a combination of substrates can influence microbial activity, moisture balance and nutrient availability. These may have accounted for the reason why WPSF-reared larvae demonstrated superior developmental performance.

The high fat content in PRWH-C may have negatively impacted larval development, preventing larvae from achieving the 50% prepupation milestone. Substrates with high fat content may induce metabolic challenges that slow progression through developmental stages. In their study, Knudsen et al. (2022) found that increasing the lipid content of substrates resulted in high mortality and low specific growth rates in larvae.

Influence of substrate type on reproductive performance of BSFL

Isnaini et al. (2023) observed that substrate type had a significant impact on egg biomass and hatchability in BSFL production. In the present study, mixed results were obtained concerning egg biomass and percentage egg hatchability across substrates. Although egg biomass did not differ significantly among substrates, notable differences were observed in percentage egg hatchability. Although hatchability rates recorded in this study were comparable to the findings of Mahmood et al. (2023), the egg biomass they obtained in their research was higher compared to what was obtained in the present study.

The findings of Morimoto et al. (2021) indicated that high-protein substrates supported rapid larval development and produced heavier adults in *Bactrocera tryoni*. This may account for the larger larval biomass observed in the offspring in PRWH-A, CF and WPSF. Additionally, the highest egg hatchability rate recorded by CF may be attributed to its enriched and balanced nutrient profile. Likewise, Mussaddeq et al. (2002) observed that commercial chicken feed exhibited superior performance in egg hatchability metrics, compared to other substrates. The lowest egg hatchability rate detected in PRWH-B may be due to the nutritional constraints imposed by its substrate composition. Water hyacinth is a substrate known for its high fibre content, and it constituted a larger proportion of PRWH-B. Nutrient assimilation during larval development may have been negatively affected due to the high fibre load in PRWH-B. This may have induced suboptimal adult conditions and subsequently resulted in lower egg viability. High-fibre substrates have been found to decrease larval development in BSFL production (Opare et al. 2024). Additionally, among the PRWH substrates, PRWH-B had the smallest proportion of para rubber seed meal, which likely resulted in low-fat levels in the substrate. Consequently, this may have lowered egg hatchability rates and larval weights observed in the offspring that emerged in PRWH-B. Petkov et al. (2022) learned that feeding birds with a high-fat BSFL meal resulted in high egg hatchability rates. Even though PRWH-C was a fat-dense substrate, its failure to produce heavy offspring may have stemmed from some anti-nutritional factors that may have persisted in the substrate post-cooking of the para rubber seeds. Pantaya et al. (2023) noted that, despite the ability of fungi to reduce the anti-nutritional component of para rubber seed meal, some factors may persist. According to Tadele (2015), anti-nutritional factors can limit nutrient utilisation in animals.

Observations on adult BSF sex ratio in this study aligned with the findings of Cai et al. (2022), who found no significant differences concerning the sex ratio of adult BSF. This was further substantiated by the findings of Putra & Safa'at (2020), who reported that rearing BSFL on a mixture of fish offal, banana skin and tofu dregs did not influence the sex ratio of emerged adults.

In contrast to this study, Singh et al. (2021) documented significant differences in BSF adult weights across three distinct organic waste streams. The substrates used in their study (fruit and vegetable mix, soy-corn-wheat bran mix and dairy manure) are likely to have a far wider nutritional diversity. This wide spectrum of nutrients may have contributed to the significant adult weight differences, whereas the comparatively similar nutrient profiles of the water hyacinth-based substrates used in the present study may have limited variations in adult body sizes.

In a study conducted by Holmes et al. (2013), prepupae were kept on sand, topsoil, wood shavings and potting soil to determine pupal duration, development and adult emergence success. The results indicated that there were no significant differences in the number of emerged adults from each substrate. Consistently, this study also found no significant differences in adult emergence counts across all substrates, which may be attributed to the relatively similar nutritional composition among substrates, due to the presence of water hyacinth in each substrate. Water hyacinth is known to contain limited fat, relatively low protein and high fibre levels. Low-fat content substrates have been found to reduce both palatability and voluntary feed intake by animals(Moses et al. 2020). The use of water hyacinth as a primary ingredient in the formulated substrates tested in this study may have imposed comparable nutritional and palatability

constraints across substrates, irrespective of the supplemental organic waste streams. This limitation may explain why the number of emerged adults was uniform across substrates.

CONCLUSION

This study examined the effects of eleven (11) water hyacinth-based diets on the growth performance of BSFL. Growth parameters, as well as some selected biological traits, were assessed. The goal was to identify the most successful diet which promoted growth, prepupation success and favourable reproductive outcomes in BSFL. The results indicated that five (5) of the eleven (11) feeding substrates that were tested were effective in that regard. These substrates were the three PRWH variants, WPSF and CF, with PRWH-A outperforming CF in terms of morphometric traits. For instance, with respect to larval weight, PRWH-A was higher than CF by 6.87%. Pig manure-hyacinth (SMWH) substrates were noted to perform sub-optimally in almost all measured parameters compared to the other substrates. Particularly, SMWH-B recorded larval weights that were 92.4% and 92.89% less than those of CF and PRWH-A, respectively. These results underscore the effects of substrate type and composition on BSFL development and biological traits, highlighting the need to prioritise thorough selection of BSFL substrates to optimise larval production. In addition, the study highlights the benefits of rearing BSFL on distinct substrate blends comprising water hyacinth, para rubber seed, fruit waste and pig manure waste streams in insect biomass production systems. Rearing BSFL on waste streams will ensure a sustainable nutrient-rich raw material for animal feed production. The results presented here hold potential for exploiting BSF for the sustainable management of water hyacinth, para rubber seed, and other waste streams to bring value to animal production.

Declarations

Competing interests

The authors declare no competing interests.

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Author Contribution

S.G.A. conceptualised the study, collected the data, performed data analysis, and wrote the manuscriptS.A.B supervised the study and critically reviewed the manuscriptM.O contributed to the study conception and critically reviewed the manuscriptES.K.O collected and processed water hyacinth and reviewed the manuscriptS.B.O contributed to data collection, data analysis and reviewed the

manuscriptF.E.A reviewed and edited the manuscriptP.K.B supervised the study and critically reviewed the manuscript

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Data Availability

The datasets generated and/or analysed during this study are available from the corresponding author upon reasonable request.

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Figures

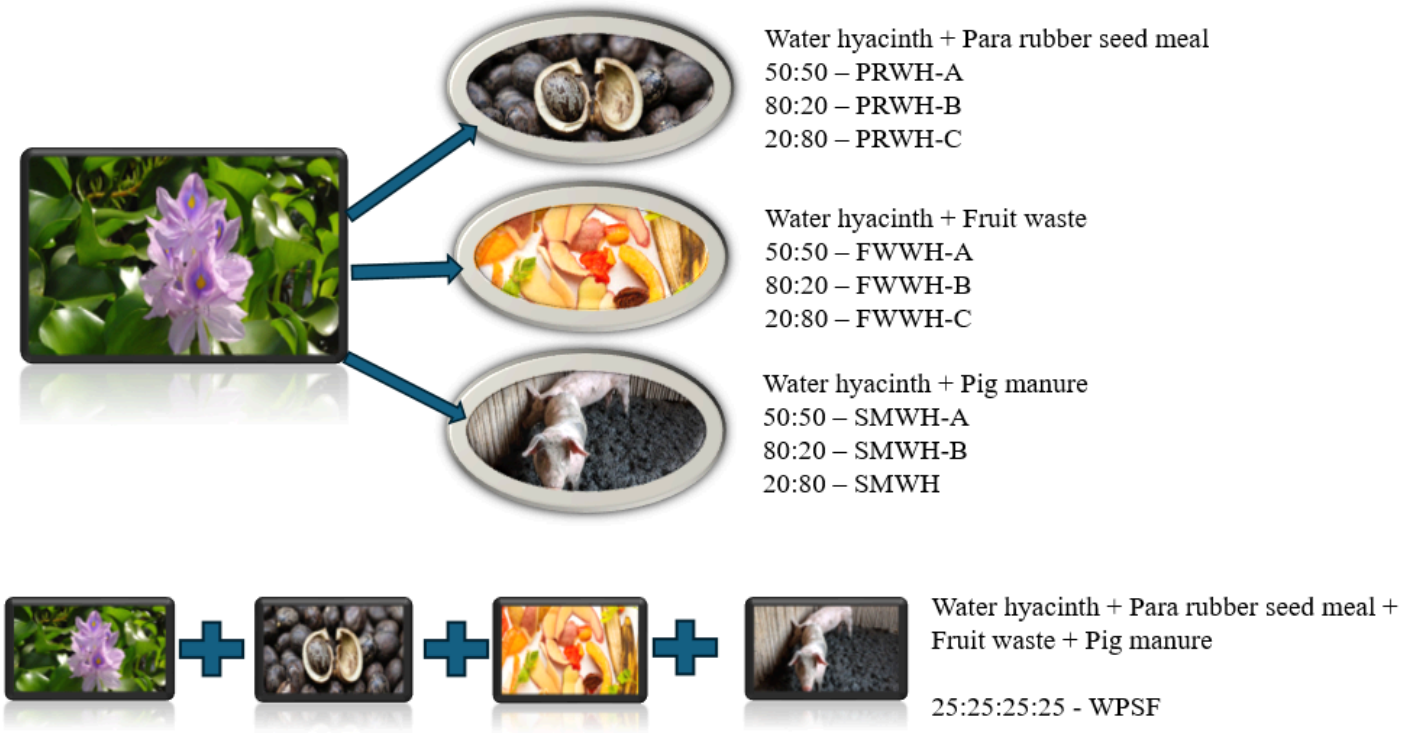


Figure 1

A graphical illustration of experimental substrates. The control diet used was chicken feed

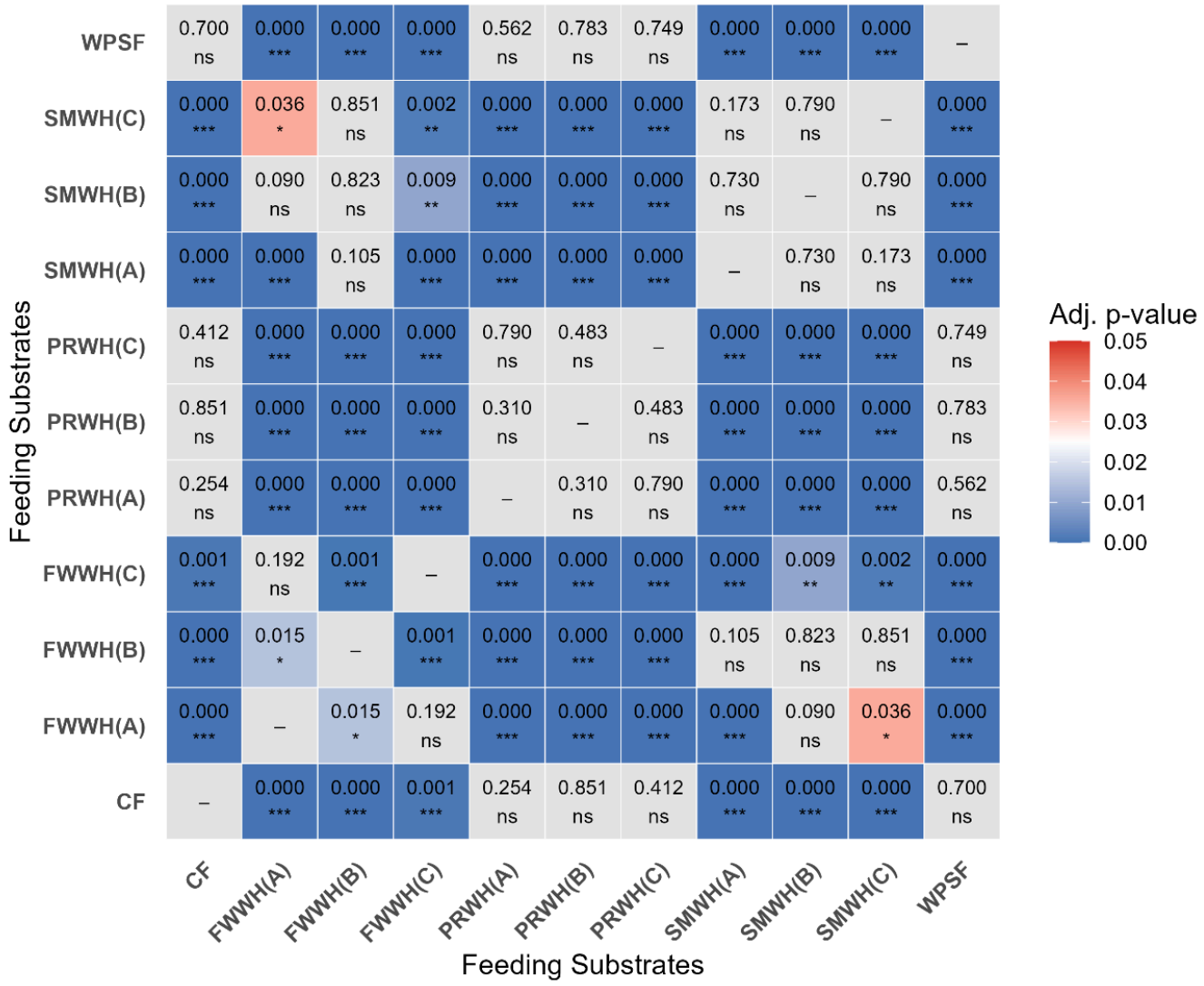


Figure 2

Fig. 1 Pairwise PERMANOVA comparisons of mean larval weight across feeding substrates.

Notes. Cells display adjusted p-values (Benjamini–Hochberg correction) with significance codes ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***; ns = not significant). PERMANOVA tests were performed using 999 permutations. Darker blue = stronger significance (Adj. $p < 0.001$); red/orange = significant at Adj. $p < 0.05$; white/light blue = nonsignificant (ns). WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

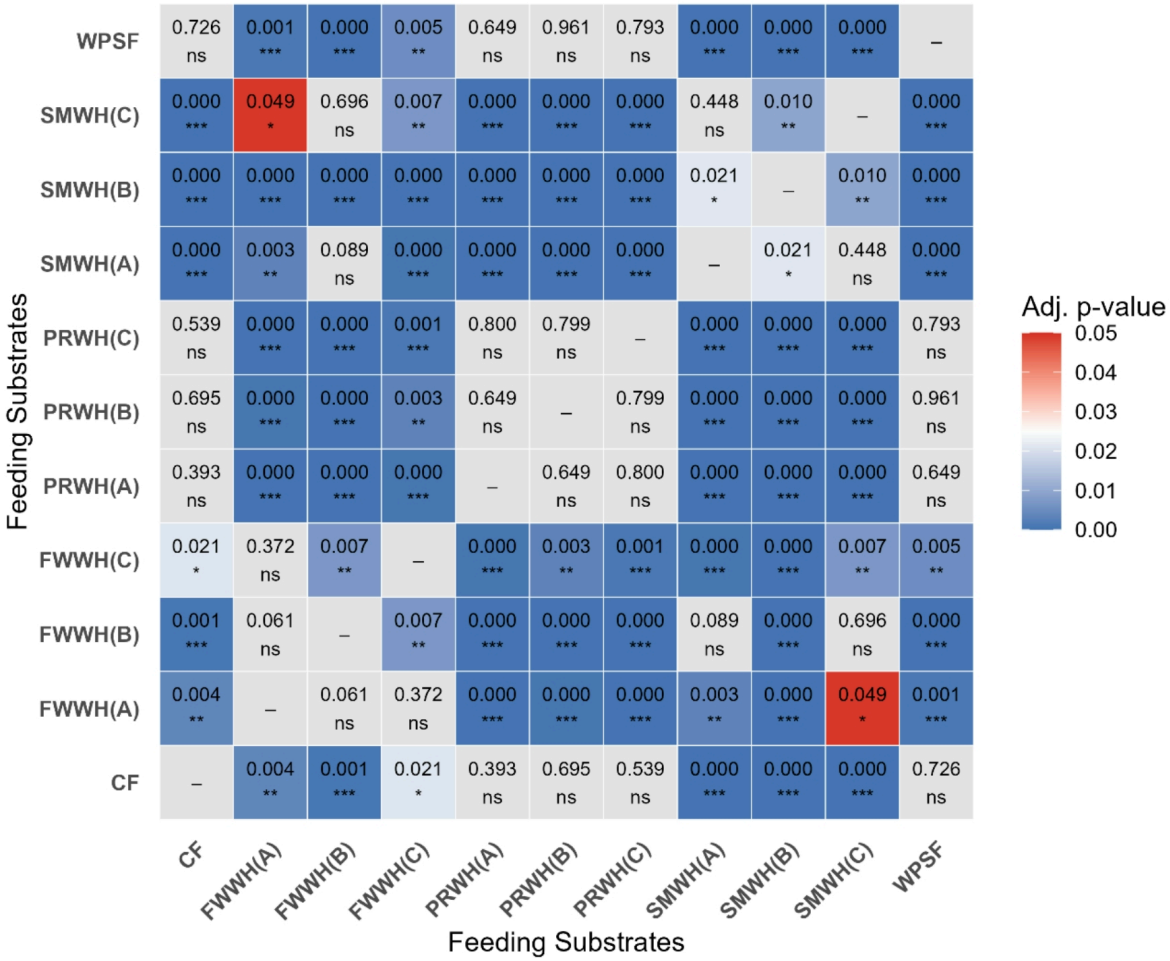


Figure 3

Fig. 2 Pairwise PERMANOVA comparisons of mean larval length across feeding substrates.

Notes. sCells display adjusted p-values (Benjamini–Hochberg correction) with significance codes (p < 0.05*, p < 0.01**, p < 0.001***; ns = not significant). PERMANOVA tests were performed using 999 permutations. Darker blue = stronger significance (Adj. p < 0.001); red/orange = significant at Adj. p < 0.05; white/light blue = nonsignificant (ns). WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

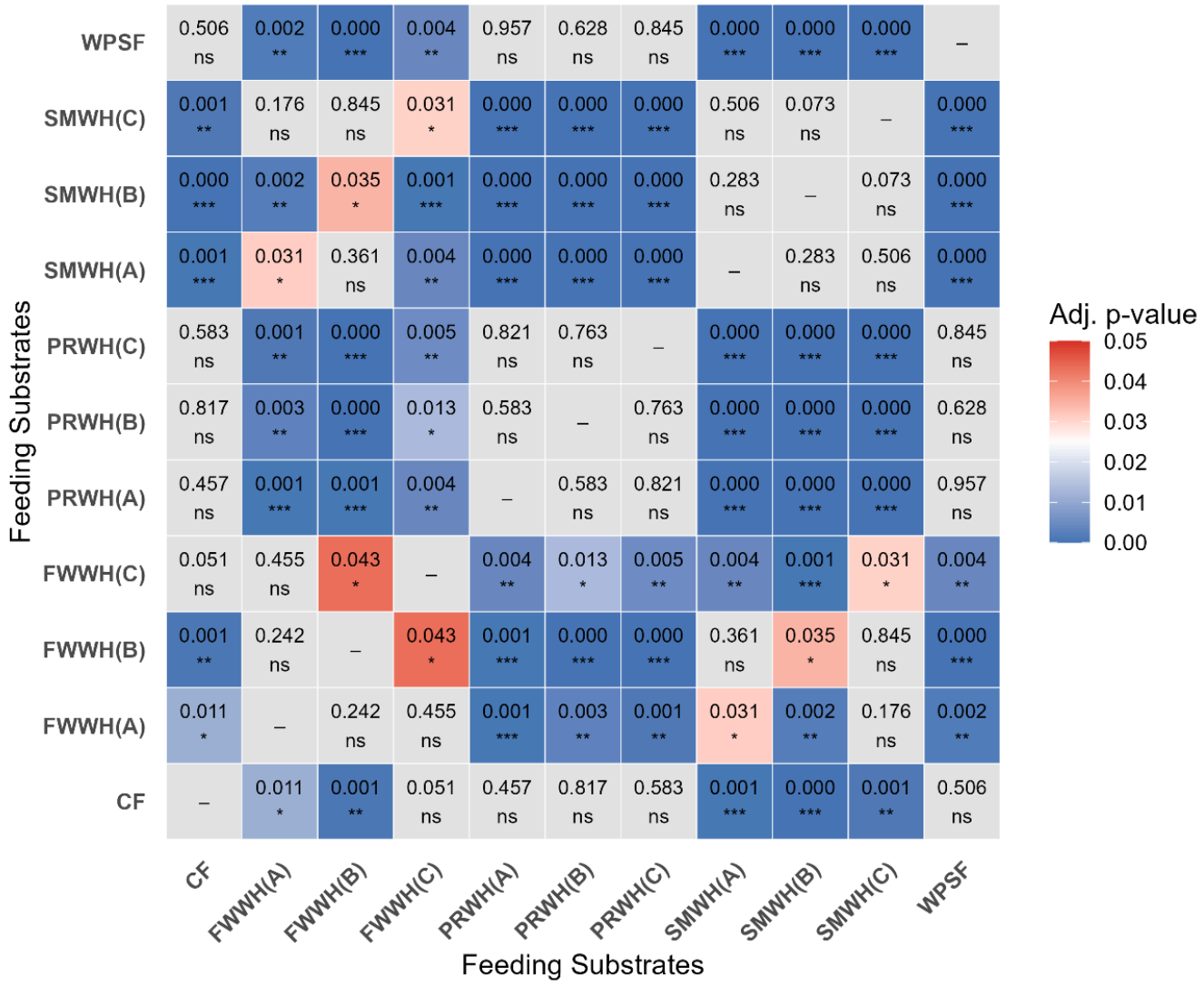


Figure 4

Fig. 3 Pairwise PERMANOVA comparisons of mean larval head capsule widths across feeding substrates.

Notes. Cells display adjusted p-values (Benjamini–Hochberg correction) with significance codes ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***; ns = not significant). PERMANOVA tests were performed using 999 permutations. Darker blue = stronger significance (Adj. $p < 0.001$); red/orange = significant at Adj. $p < 0.05$; white/light blue = nonsignificant (ns). WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

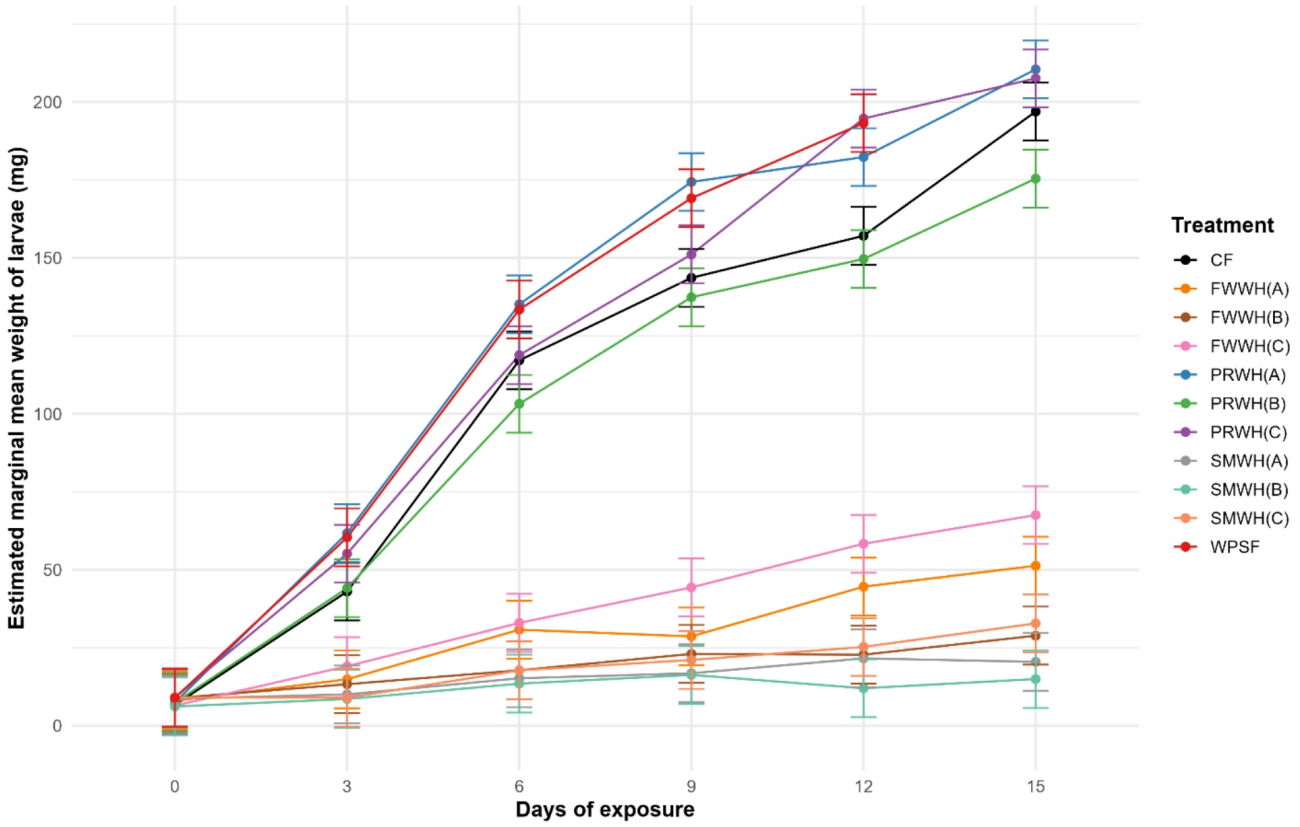


Figure 5

Fig. 4 The effects of substrate composition and period of exposure on the mean weight of BSFL

Notes. Estimated marginal means of BSFL weight (mg) over time. Estimated marginal means (± SE) of larval weight across days and feeding substrates derived from a robust linear model. Pairwise comparisons were performed using the emmeans package with Tukey adjustment at p = 0.05. Higher trajectories indicate faster morphological development across feeding substrates. WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

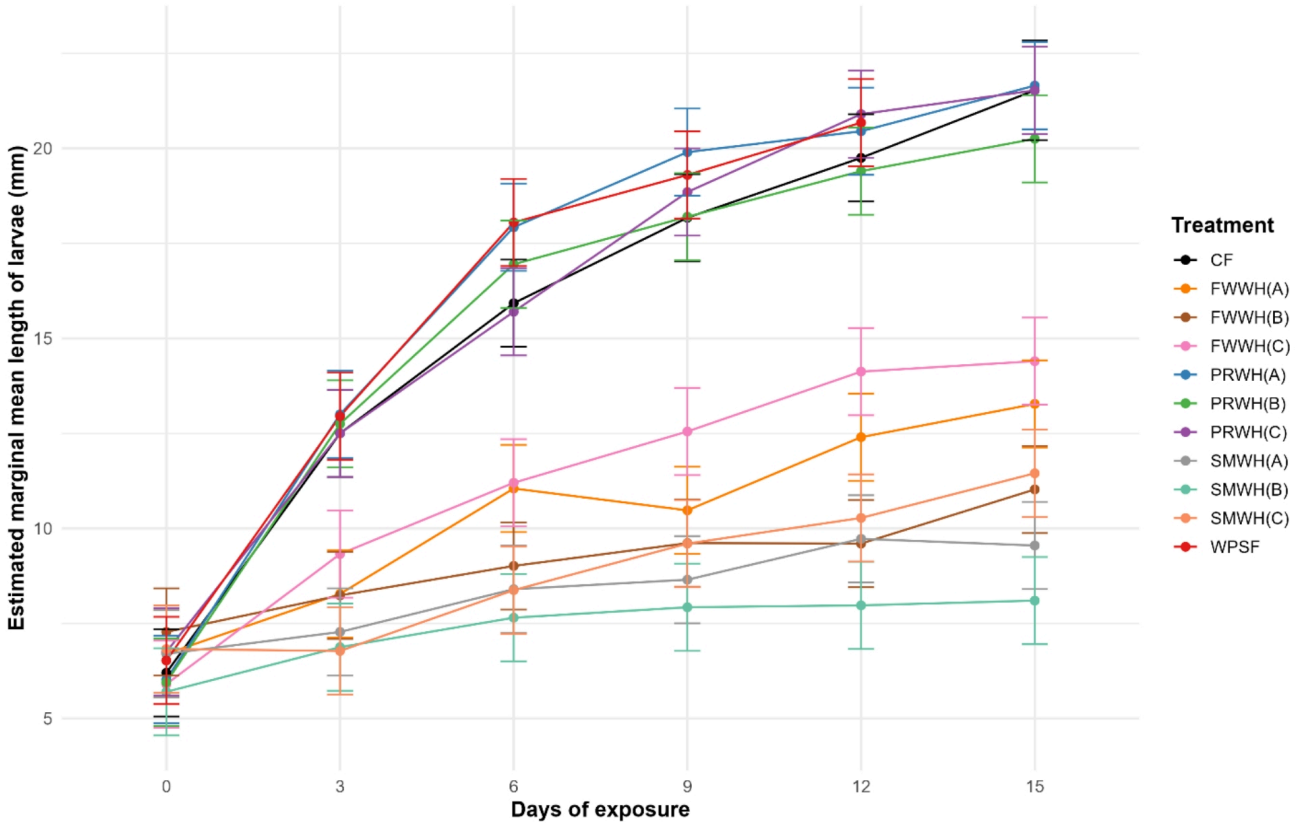


Figure 6

Fig. 5 The effects of substrate composition and period of exposure on the mean length of BSFL

Estimated marginal means of BSFL length over time. Estimated marginal means (± SE) of larval length across days and feeding substrates derived from a robust linear model. Pairwise comparisons were performed using the emmeans package with Tukey adjustment at p = 0.05. Higher trajectories indicate faster morphological development across feeding substrates. WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

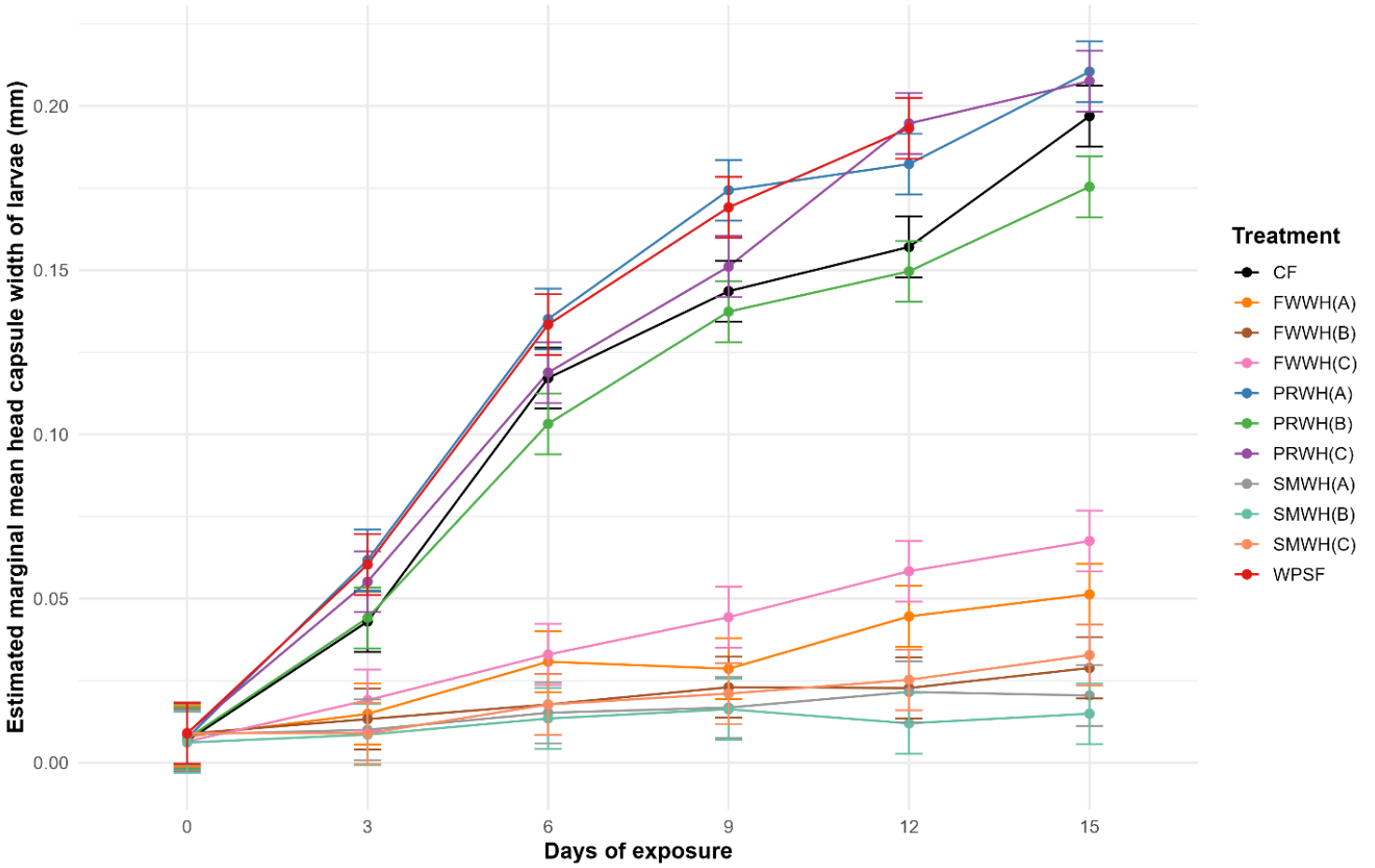


Figure 7

Fig. 6. Effects of substrate composition and period of exposure on the mean head capsule width of BSFL over time

Pairwise comparisons were performed using the emmeans package with Tukey adjustment at p = 0.05. Higher trajectories indicate faster morphological development across feeding substrates. WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH(C) = 80:20 pig manure to water hyacinth, SMWH(B) = 20:80 pig manure to water hyacinth, SMWH(A) = 50:50 pig manure to water hyacinth, PRWH(C) = 80:20 milled para rubber seed to water hyacinth, PRWH(B) = 20:80 milled para rubber seed to water hyacinth, PRWH(A) = 50:50 milled para rubber seed to water hyacinth, FWWH(C) = 80:20 fruit waste to water hyacinth, FWWH(B) = 20:80 fruit waste to water hyacinth, FWWH(A) = 50:50 fruit waste to water hyacinth, CF = chicken feed

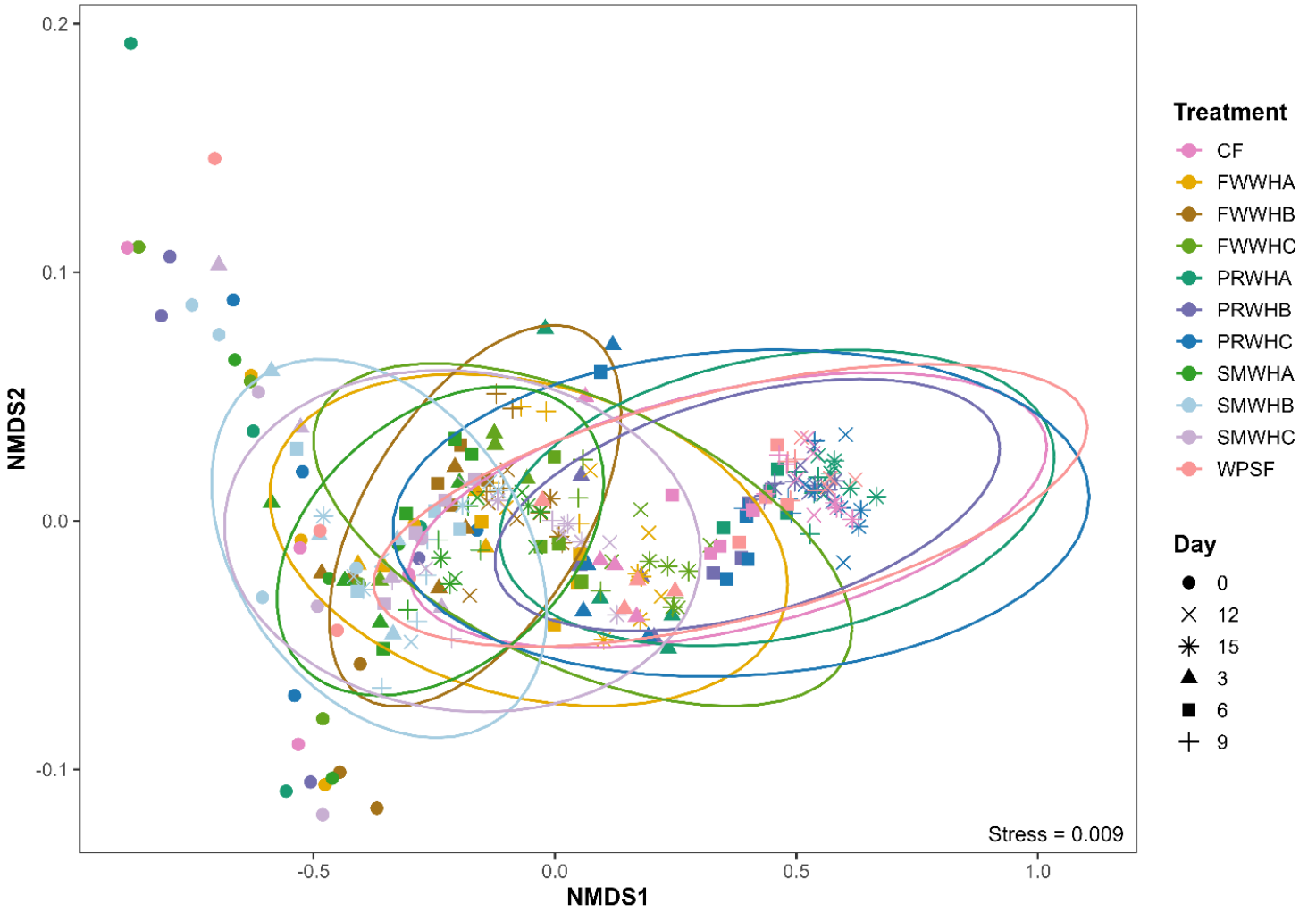


Figure 8

Fig. 7 Non-metric multidimensional scaling (NMDS) ordination (stress = 0.009) illustrating multivariate differences in BSFL morphometric traits across feeding substrates and sampling days

Ellipses represent 95% confidence regions around treatment centroids. Analysis was performed using PERMANOVA (999 permutations) with a significance threshold of p = 0.05. WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWHC = 80:20 pig manure to water hyacinth, SMWHB = 20:80 pig manure to water hyacinth, SMWHA = 50:50 pig manure to water hyacinth, PRWHC = 80:20 milled para rubber seed to water hyacinth, PRWHB = 20:80 milled para rubber seed to water hyacinth, PRWHA = 50:50 milled para rubber seed to water hyacinth, FWWHC = 80:20 fruit waste to water hyacinth, FWWHB = 20:80 fruit waste to water hyacinth, FWWHA = 50:50 fruit waste to water hyacinth, CF = chicken feed

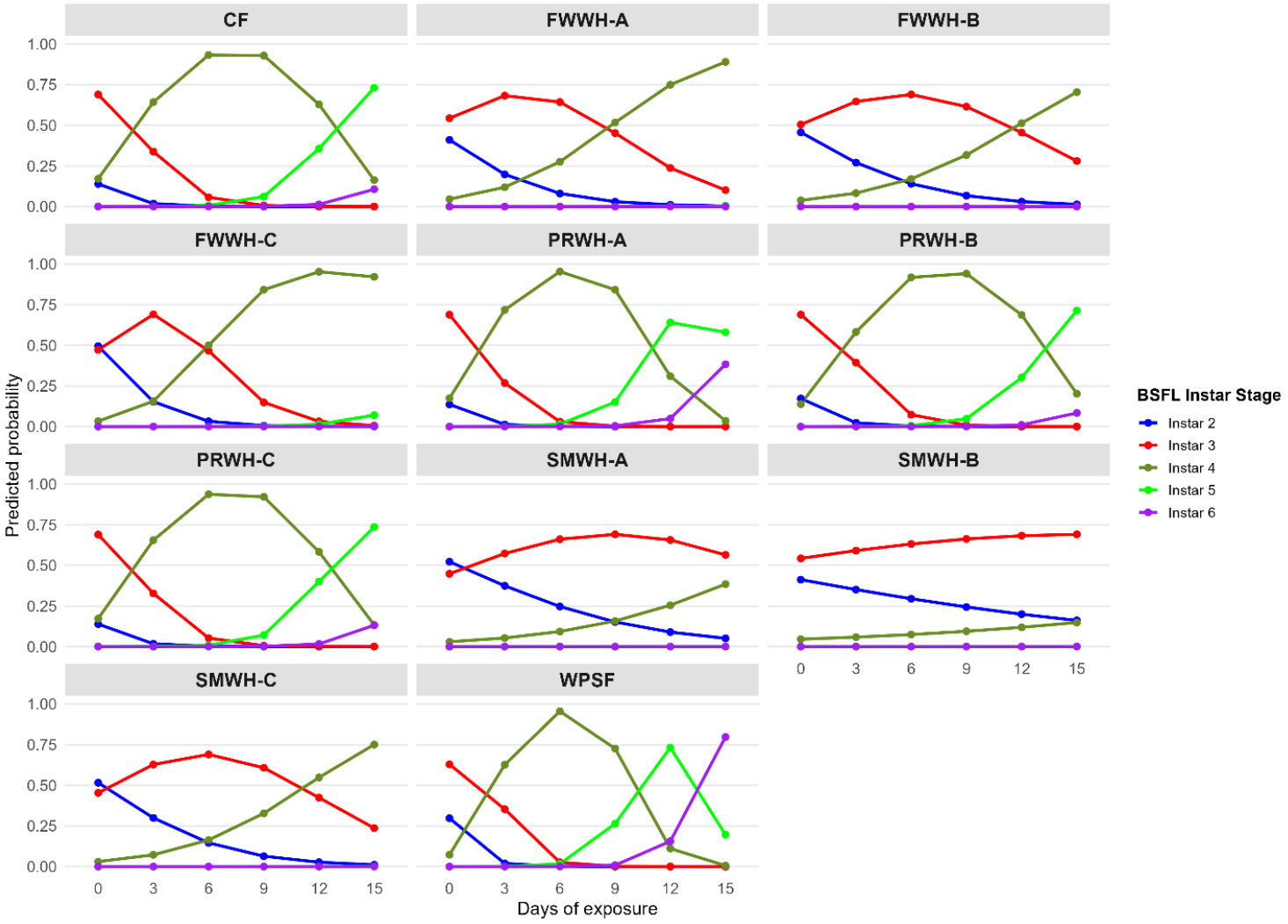


Figure 9

Fig. 8 Predicted instar-stage probabilities (0–15 days of exposure) across rearing substrate**s**

Panels show predicted probabilities for instars 2–6 across sampling days for each substrate; multinomial logistic regression (glm) with substrate and day as predictors; pairwise comparisons adjusted using the Tukey method (p = 0.05). WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH-C = 80:20 pig manure to water hyacinth, SMWH-B = 20:80 pig manure to water hyacinth, SMWH-A = 50:50 pig manure to water hyacinth, PRWH-C = 80:20 milled para rubber seed to water hyacinth, PRWH-B = 20:80 milled para rubber seed to water hyacinth, PRWH-A = 50:50 milled para rubber seed to water hyacinth, FWWH-C = 80:20 fruit waste to water hyacinth, FWWH-B = 20:80 fruit waste to water hyacinth, FWWH-A = 50:50 fruit waste to water hyacinth, CF = chicken feed

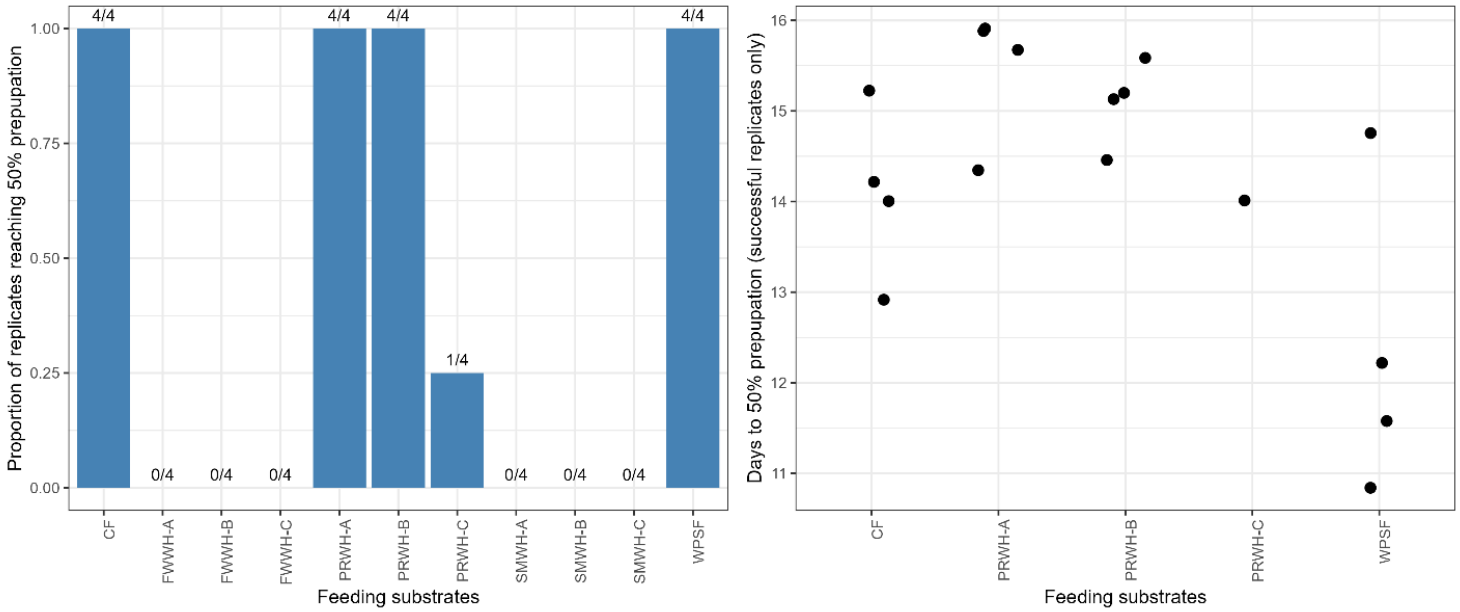


Figure 10

Fig. 9 Substrate Effects on Time to 50% Prepupation and Replicate Success in BSFL

Proportion of substrates reaching 50% prepupation (left) and days to 50% prepupation for successful replicates only (right) across feeding substrates. Bars show the number of successful replicates out of four per treatment. Differences among substrates were evaluated using one-way ANOVA (p = 0.05) for the number of days to 50% prepupation; treatments failing to reach 50% prepupation were excluded from ANOVA and plotted descriptively only. WPSF = water hyacinth, milled para rubber seed, pig manure and fruit waste in a 25:25:25:25 ratio, SMWH-C = 80:20 pig manure to water hyacinth, SMWH-B = 20:80 pig manure to water hyacinth, SMWH-A = 50:50 pig manure to water hyacinth, PRWH-C = 80:20 milled para rubber seed to water hyacinth, PRWH-B = 20:80 milled para rubber seed to water hyacinth, PRWH-A = 50:50 milled para rubber seed to water hyacinth, FWWH-C = 80:20 fruit waste to water hyacinth, FWWH-B = 20:80 fruit waste to water hyacinth, FWWH-A = 50:50 fruit waste to water hyacinth, CF = chicken feed