FLY LARVAE FOR AQUAFEED

This Food Agility CRC project sought to improve black soldier fly larvae production and product quality assessment for aquaculture.

By Mikaeylah Davidson, Jessica Maddams, Talukdar Jannat Tamanna, Leo Nankervis, Andreas Lopata, Bronson Philippa & Kyall Zenger

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PROJECT DESCRIPTION

This project aimed to improve black soldier fly larvae (BSFL) production and product quality assessment for aquaculture applications through the development of rapid diagnostic tools and new compositional datasets. Specifically, the project sought to evaluate the capability of Near Infra-Red (NIR) spectroscopy to provide rapid, real-time assessment of the nutritional composition and quality of live and processed BSFL reared on different waste stream-derived diets, reducing reliance on slow and costly laboratory analyses. The project also aimed to characterise BSFL protein and antimicrobial peptide (AMP) composition and evaluate the effects of BSFL-based aquafeeds on prawn growth, survival, and health-related outcomes. Collectively, these outcomes were intended to support improved production management, product standardisation, and informed adoption of BSFL ingredients within aquaculture systems.

PROJECT PARTNERS

This project was a partnership between:

- James Cook University (Research Partner)
- FlyFarm Queensland (Industry partner)
- Tassal (Industry partner)
- Food Agility CRC (Innovation Partner)

EXECUTIVE SUMMARY

BACKGROUND

The black soldier fly larvae (*Hermetia illucens*) (BSFL) industry is rapidly expanding as a sustainable alternative for converting organic waste streams into high-value protein and bioactive ingredients for use in animal feed. However, continued industry growth requires improved understanding of BSFL product quality, nutritional consistency, functional properties and applications within commercial feed systems.

Variation in BSFL production conditions, including rearing substrates, processing methods and environmental conditions, can influence the nutritional and functional composition of larval products. This creates challenges for product standardisation, quality assurance and end-user confidence. Additionally, while BSFL meal (BSFLM) has demonstrated potential as a sustainable feed ingredient, further research is required to understand how production and processing conditions influence functional properties and how it performs within aquaculture production systems.

This project addressed these challenges by developing rapid quality assessment approaches, characterising the nutritional and bioactive composition of larval products, and evaluate its inclusion as an aquafeed ingredient for black tiger prawn (*Penaeus monodon*) production.

PROJECT AIMS AND OBJECTIVES

The project aimed to improve industry understanding of BSFL product quality, functional composition and feed applications through three integrated objectives:

1. **Develop rapid quality assessment tools for BSFL products**
   Develop and validate near-infrared (NIR) spectroscopy for rapid prediction of BSFL protein and lipid composition to support product standardisation and quality control.
2. **Characterise BSFL protein and antimicrobial peptide (AMP) composition**
   Investigate how industrial processing and rearing substrate influence the BSFL proteome and antimicrobial peptide profiles.
3. **Evaluate BSFL as an aquafeed ingredient for marine shrimp**
   Assess the effects of BSFLM inclusion in black tiger prawn diets on growth performance and intestinal microbial communities.

METHODOLOGY

The project combined analytical chemistry, proteomics, bioinformatics and aquaculture feeding trials to evaluate BSFL products from production through to end-use applications.

Rapid quality assessment of BSFL products

NIR spectroscopy was used to assess the ability to rapidly predict nutritional composition from live and processed BSFL samples. Predictive models were developed using reference compositional analyses and validated using independent production cycles to assess model robustness.

BSFL protein and bioactive composition

High-resolution liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to characterise BSF protein composition and identify immune-associated proteins and antimicrobial peptide candidates. Custom bioinformatic workflows were developed to analyse BSF immune proteins and assess how processing and rearing feedstocks influence bioactive composition.

Application of BSFL in aquafeeds

A controlled feeding trial was conducted in black tiger prawns to evaluate dietary inclusion of BSFLM as a replacement for conventional protein ingredients. Growth performance, survival, intestinal and hepatopancreas morphology, and intestinal microbial communities were assessed across different inclusion levels.

KEY RESULTS AND OUTCOMES

Rapid quality assessment of BSFL products

NIR spectroscopy demonstrated strong potential as a rapid quality assessment tool for processed BSFL products. Protein prediction models developed for BSFLM achieved high predictive accuracy and remained robust when applied to an independent production cycle generated under different seasonal conditions. This demonstrates the potential for NIR-based approaches to support rapid compositional assessment, quality assurance and product standardisation within commercial BSFL production systems.

Prediction of lipid composition was also promising during initial model development; however, reduced performance during independent validation highlighted the need for broader calibration datasets incorporating additional biological and production variation.

BSFL protein and bioactive composition

Proteomic analysis demonstrated that industrial processing influenced the detectable protein profile of BSF products, including reductions in immune-associated proteins and AMP candidates. This highlights the importance of considering processing effects when developing BSFL derived functional ingredients.

Rearing feedstock also influenced immune-associated protein composition. While overall immune protein richness remained similar among feedstocks, functional composition

differed, with variation observed in immune effector proteins and AMP families. These results demonstrate that BSFL production conditions provide opportunities to influence the functional characteristics of resulting products.

Application of BSFL in aquafeeds

Feeding trials demonstrated that BSFLM could replace a substantial proportion of conventional protein ingredients in black tiger prawn diets without compromising growth performance. The 50% replacement treatment produced the most favourable combination of growth performance, tissue health and intestinal microbial composition, while higher inclusion levels showed a reduction in survival.

Dietary BSFLM altered intestinal microbial community composition, demonstrating potential effects beyond nutritional replacement alone. These findings support continued evaluation of BSFLM as both a sustainable protein ingredient and a potential functional feed component.

INDUSTRY IMPACT AND BENEFICIARIES

The outcomes of this project provide direct benefits to the emerging BSFL industry by improving understanding of product quality, processing effects and feed applications. BSFL producers will benefit from improved tools for rapid quality assessment and opportunities to develop more consistent, value-added products. Feed manufacturers and aquaculture producers will benefit from improved knowledge regarding BSFLM inclusion strategies and the potential functional properties of BSFL derived ingredients. The broader agriculture and aquaculture sectors may benefit through increased availability of sustainable alternative protein ingredients, reducing reliance on conventional feed resources.

RECOMMENDATIONS

- NIR spectroscopy should be further developed and validated across additional production conditions to support commercial implementation for rapid quality assessment of dried BSFL products.
- Standardised compositional profiling of BSFL products using NIR should be encouraged to improve product consistency, end-user confidence and development of higher-value applications.
- Further optimisation of BSFL production conditions should consider nutritional quality alongside bioactive composition to enable development of targeted functional feed ingredients.
- Validate the biological activity, stability and bioavailability of BSFL proteins and AMPs to support translation into functional feed applications.

- Further investigation of BSFLM-induced changes in intestinal microbial communities should be undertaken to determine their functional significance, including potential impacts on immune function, disease resistance and resilience under commercial production conditions.
- Support continued collaboration between insect producers, aquaculture companies, technology providers and researchers to progress project outputs toward commercial implementation.

CONCLUSION

This project has demonstrated the potential for BSFL products to contribute to sustainable circular bioeconomy systems through improved quality assessment, enhanced understanding of functional composition and validated feed applications. The integration of rapid analytical tools, molecular characterisation and aquaculture performance testing provides industry with new knowledge and pathways to support the commercial development of BSFL derived feedstock.

INDUSTRY IMPACT METRICS

The impact of this project for BSFL farming and aquaculture industries will be achieved through three primary application pathways, assuming continued validation and uptake of the technologies and knowledge generated.

Impact metric 1

Reduced reliance on laboratory-based compositional testing in BSF production
The development of NIR spectroscopy approaches provides a pathway for real-time assessment of BSFL total protein and lipid composition compared to conventional laboratory methods, which are typically slow (~1–3 weeks) and costly (~$150 per sample). This improves the potential for more timely decision-making in BSFL production systems, particularly for routine product quality control and diversification.

Impact metric 2

Improved baseline understanding of BSFL functional components
The project established comprehensive protein and AMP compositional profiles for raw and processed BSFL products, addressing key knowledge gaps surrounding insect-derived functional bioactive compounds. Production variables, including feedstock selection and processing, influence the bioactive protein profile, indicating that bioactive composition is responsive to production conditions rather than being a fixed characteristic of BSFL biomass. This provides a reference point for future research into the nutritional and functional value of insect-derived ingredients.

Impact metric 3

Reduced uncertainty in the use of BSFL in aquaculture feeds
Controlled feeding trials provide empirical evidence on the potential effects of BSFL inclusion in prawn diets, including impacts on growth performance, survival, nutritional

composition, gut health, and overall animal health. This contributes to a more evidence-based foundation for evaluating BSFL as a partial replacement for conventional feed ingredients such as fishmeal and poultry meal, or as an additive ingredient.

IMPACT STATEMENTS

INDUSTRY PARTNER

Tim Shepherd, Tassal, Head of Smartfarm - Planning and Implementation

This project provides valuable new knowledge and capability to support the evaluation of BSFL as a sustainable alternative protein source in aquafeed. The outcomes contribute to ongoing efforts to identify feed ingredients that may improve sustainability, reduce reliance on traditional marine-based proteins, and support more resilient aquaculture production.

The results support the use of BSFL circular economy ingredient, where organic waste streams can be converted into high-value protein for aquafeed applications. This has potential relevance for improving the environmental footprint of feed production systems while maintaining or enhancing production performance.

The project also provides early evidence that BSFL may contain bioactive compounds that could contribute to improved prawn growth and health outcomes. While further validation is required, this supports future consideration of BSFL not only as a bulk protein replacement but also as a potential functional feed additive.

Overall, the project strengthens the knowledge base for assessing alternative protein sources and provides foundational data to inform future decisions around sustainable feed development and innovation in prawn aquaculture.

INDUSTRY PARTNER

Constant Tedder, FlyFarm, CEO

Insect farming is an emerging but rapidly growing global industry, with increasing demand for scalable production systems that can consistently deliver high-quality protein ingredients for feed applications. A key challenge for the sector is maintaining product quality while increasing production scale and diversifying product streams.

The development of near-infrared (NIR) approaches offers a pathway for rapid and lower-cost assessment of BSFL quality compared to traditional laboratory testing. This enables faster feedback on production outcomes and supports more responsive decision-making in on-farm management. In addition, the project contributes to improved understanding of

how both larval diet and post-harvest processing influence the nutritional and functional composition of BSFL, including protein and antimicrobial peptide profiles. These findings support opportunities for product diversification, optimisation of production processes, and the development of new value-added insect-based products.

Overall, the outcomes support improved product quality management and explore new product development opportunities. The project aligns with broader industry goals of scaling sustainable insect production systems and strengthening circular economy approaches through the conversion of organic inputs into consistent, high-quality protein ingredients.

RESEARCH PARTNER

Professor Kyall Zenger, Senior academic, James Cook University

This project has strengthened research capability in the development and application of sustainable insect-derived feed ingredients by integrating BSFL production, analytical characterisation and aquaculture performance evaluation. The research has generated new knowledge on how production conditions, processing approaches and product composition influence the nutritional and functional characteristics of BSFL ingredients, providing evidence to support their future application within circular economy-based aquafeed.

The project has also supported the training of the next generation of scientists through multidisciplinary PhD research spanning aquaculture nutrition, insect production, proteomics, bioinformatics and molecular characterisation. Researchers involved in the project have developed technical expertise and experience in translating scientific findings into industry relevant outcomes.

The project has expanded our scientific understanding of how organic waste streams can be converted into higher-value biological resources for production systems. By combining expertise across multiple disciplines, the project has established approaches for evaluating alternative feed ingredients and generated knowledge to support future research and industry collaboration.

Collectively, the outcomes contribute to the development of more sustainable aquaculture production systems, improved resource utilisation and the advancement of circular economy approaches within the broader animal food production sector.

INNOVATION PARTNER

Food Agility CRC
Dave Lamb and Lucy Hickman

At a time when industries are increasingly focussed on ways to reduce their ecological footprint and become more sustainable, the Fly Larvae for Aquafeed project has showcased how even the smallest creatures have the potential to make a big impact.

By focussing on Black Soldier Fly Larvae as an alternative feedstock, the project team has developed and validated advanced diagnostic approaches, including near-infrared (NIR) technologies capable of linking BSFL nutritional composition with different waste-stream inputs and production processes as well as used modelling to predict certain characteristics.

By partnering with industry partners FlyFarm and Tassal, the project has assessed the impact of this alternative feed on prawn growth and survival. This collaboration has been greatly beneficial in validating the research and creating a real-world use case for the BSF. Overall, this project has set up a strong foundation for future research, development and commercialisation of the insect protein industry and it is exciting to see how it will continue to contribute to advancements in the aquafeed industry.

END-USER PROFILE

Tim Shepherd, Tassal

Tim leads Tassal's Smartfarm division, supporting the evaluation and implementation of new technologies and production approaches across aquaculture systems. For prawn farming, feed performance is a major driver of productivity, cost efficiency, and sustainability. As aquaculture industries continue to seek sustainable alternatives to conventional feed ingredients such as fishmeal and poultry products, there is increasing interest in circular economy strategies, such as using BSFL. However, uncertainty around feed performance and ingredient consistency presents a challenge for industry adoption.

For Tassal, understanding whether alternative feed ingredients can maintain or improve prawn growth, survival, and overall production performance is essential before integration into commercial systems. This project provides practical evidence on how BSFL-based feeds perform under controlled trial conditions and improves understanding of the nutritional quality of insect-derived ingredients.

"A major challenge is balancing performance, cost, and sustainability in feed formulation. We need confidence that alternative ingredients perform consistently in real production systems."

The project also demonstrates rapid quality assessment approaches for BSFL ingredients through NIR technologies, which may support faster feed ingredient evaluation in the future.

"Having stronger evidence around feed performance helps reduce uncertainty when assessing new ingredients and supports more informed decisions for aquafeed development."

OBJECTIVES

This project investigated approaches to improve BSFL production, product quality assessment, and aquaculture feed applications through rapid diagnostics, compositional profiling, and prawn feeding trials.
Project objectives included:

1. Develop NIRs diagnostic capability to rapidly measure on-farm BSFL composition and BSFL product quality in real-time.
2. Assess BSFL protein and AMP composition from raw and processed BSFL products.
3. Evaluate effects of BSFL supplemented aquafeed on marine shrimp growth and survival.

METHODOLOGY

OVERVIEW OF EXPERIMENTAL DESIGN

The project was structured around three primary objectives, with distinct but integrated methodological approaches applied to each. These methods are outlined in the sections below.

NIR SPECTROSCOPY DEVELOPMENT

BSFL were produced across three dietary protein treatments (low 11%, medium 30%, and high 50%) to generate systematic variation in nutritional composition. Larvae were harvested once approximately 50% reached instar five, with samples collected across multiple grow-out trays to capture within-colony biological variation.

NIR spectroscopy was used to evaluate the ability to rapidly predict BSFL protein and lipid composition. Spectral data were collected from both live larvae immediately post-harvest (N = 513) and from processed, microwave-dried larvae that were homogenised into a fine black soldier fly meal (BSFM) and analysed as batched samples (N = 106). Following NIR analysis, live larvae were humanely euthanised and stored at -80 °C, while BSFM samples were stored at 4 °C prior to further compositional analysis.

Reference analyses were undertaken to quantify nutritional composition and provide calibration datasets for model development and validation. Crude protein content of individual live larvae was determined using a Bradford colorimetric assay. For BSFM, protein content was quantified using the Kjeldahl method with a nitrogen-to-protein conversion factor of 4.43, lipid content was determined using Soxhlet extraction with hexane, and ash content was measured by combustion in a muffle furnace at 550 °C for 8 hours.

NIR spectral data were linked to reference composition data to develop predictive models of BSFL nutritional quality in Matlab 2025b (Mathworks). The raw spectra were pre-processed using Savitsky-Golay derivatives, with calibration models developed using partial least squares regression, Gaussian process regression and support vector machine regression. Model performance was assessed using cross-validation approaches to evaluate predictive accuracy and robustness across dietary treatments and sample types.

To assess model generalisability, predictive models developed from the original production cycle were applied to an independent validation dataset generated from BSFL reared under the same dietary treatments in a separate production cycle five generations later and under different seasonal conditions. This enabled assessment of model transferability across biological and environmental variation without recalibration.

BSFL PROTEIN AND AMP CHARACTERISATION

To characterise the protein and antimicrobial peptide (AMP) composition of raw and industrially processed BSFL, a processing experiment was conducted using larvae reared on a commercial diet under controlled conditions. Five paired biological replicates were collected from the same production batch, with approximately 500 larvae sampled for each replicate prior to processing. Each replicate was divided into matched subsamples, with one subsample freeze-dried to represent the raw (pre-drying) condition and the second subjected to proprietary industrial drying to represent the processed (post-drying) condition. All samples were harvested at a consistent developmental stage and prepared and stored using standardised protocols prior to analysis.

To further investigate factors influencing BSFL protein and AMP composition, an additional experiment examined the effects of rearing feedstock. BSFL were reared under controlled conditions on three waste-derived feedstocks (Fruit and Vegetable waste (FVW), Soy Okara (SO) and Brewer's Spent Grain (BSG). Each dietary treatment comprised of three independent biological replicate trays containing approximately 1,500 larvae. Following harvest, 200 larvae were collected from each replicate to generate a representative sample for proteomic analysis.

Proteins were extracted using a urea/SDS based lysis buffer, followed by sonication, centrifugation, protein quantification and S-Trap sample preparation with trypsin digestion. Peptides were analysed by high resolution liquid chromatography tandem mass spectrometry (LC-MS/MS). Raw mass spectrometry data were processed in MaxQuant (v2.1.0.0) against the *Hermetia illucens* UniProt reference proteome, with standard filtering to generate protein identification datasets.

Bioinformatic analyses were undertaken to characterise the whole proteome, immune associated proteins and AMP candidates. As comprehensive annotation workflows for immune proteins and AMPs are not currently available for *H. illucens* a dedicated bioinformatic pipelines was developed and implemented in Python (v3.8.10). The pipeline integrated Gene Ontology (GO) annotation, protein name and keyword screening, protein domain information, and comparisons with literature curated AMP reference databases to systemically identify and classify immune associated proteins and AMP candidates. Candidate AMPs were subsequently assigned to recognised AMP families where sufficient supporting evidence was available.

Statistical analyses were conducted in Python. Differences in protein richness and AMP candidate abundance among dietary treatments were assessed using Kruskal-Walis, while paired Wilcoxon signed rank tests were used to evaluate the effects of industrial processing. Multivariate differences in immune protein functional composition were assessed using Bray-Curtis dissimilarities, principal coordinates analysis (PCoA), permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersion (PERMDISP).

BSFL INCORPORATED AQUAFEED EFFECT ON PRAWN GROWTH AND SURVIVAL

To assess the effects of BSFLM incorporation into aquafeeds, a feeding trial was conducted to evaluate impacts on growth performance, intestinal and hepatopancreas health, and gut microbial communities of black tiger prawns (*Penaeus monodon*).

Dried BSFL were provided by our project partner Fly Farm and samples were partially defatted prior to incorporation into experimental diets. Four isonitrogenous diets (46% crude protein) were formulated, consisting of a control diet containing fish meal and poultry by-product meal, and three experimental diets where poultry by-product meal was progressively replaced with partially defatted BSFLM at 25%, 50% and 75% replacement levels.

A single-factor feeding trial was conducted using 600 juvenile prawns (PL60) distributed among 20 experimental tanks, with five replicate tanks per dietary treatment. Following

acclimation, prawns were stocked at 30 individuals per tank and maintained under controlled environmental conditions. Prawns were fed the experimental diets four times daily, with feeding rates adjusted based on biomass and feed intake throughout the trial period.

Following completion of the feeding trial, prawns were fasted for 24 hours before assessment of production performance. Growth and health parameters including final body weight, weight gain, specific growth rate, survival, total body length, condition factor and hepatosomatic index were calculated. Hepatopancreas and intestinal tissues were collected for histological and molecular analyses. Histological assessment of hepatopancreas and intestinal morphology was conducted, with measurements of intestinal villus height and mucosal thickness used to assess dietary effects on gut health.

Intestinal microbial communities were characterised using full-length 16S rRNA gene amplicon sequencing. DNA extraction, PCR amplification and sequencing were conducted by the Australian Genome Research Facility using PacBio HiFi sequencing technology. Sequence data were processed using QIIME2 and DADA2 workflows, with taxonomic classification performed against curated microbial reference databases. Alpha and beta diversity analyses were conducted to assess differences in microbial community richness, diversity and structure among dietary treatments.

Statistical analyses were performed to evaluate dietary effects on growth performance, tissue morphology and microbial community composition. Growth and histological parameters were assessed using ANOVA with post-hoc comparisons, while microbial community differences were evaluated using diversity metrics, Bray-Curtis dissimilarity, PCoA, PERMANOVA and tests of multivariate dispersion.

RESULTS

NIR SPECTROSCOPY DEVELOPMENT

Dietary protein treatments successfully generated differences in BSFL nutritional composition, with larvae reared on higher protein diets generally exhibiting greater protein concentrations than those reared on lower protein diets. Variation in larval composition across treatments confirmed that feed inputs influenced BSFL nutritional quality and provided sufficient compositional diversity for NIR model development.

Prediction performance differed substantially between live larvae and processed dried BSFM. For live larvae, NIR spectroscopy demonstrated limited ability to accurately predict protein content (Figure 1a). While broad compositional trends among dietary groups were evident, predictive performance across models remained low ($R^2 < 0.30$), indicating that nutritional composition of live larvae could not be reliably estimated under the conditions tested. The lower performance observed in live larvae was likely influenced by biological variability among individuals, moisture content, and light penetration through the chitin exoskeleton affecting spectral consistency.

In contrast, strong predictive performance was achieved for processed dried BSFM (Figure 1b). Protein prediction demonstrated high accuracy across all modelling approaches, with models explaining approximately 85–91% of variation in measured protein composition (R^2 = 0.848–0.906). Prediction error remained low, and model performance metrics indicated strong agreement between measured and predicted values, demonstrating the suitability of NIR for estimating protein content in dried BSFM products.

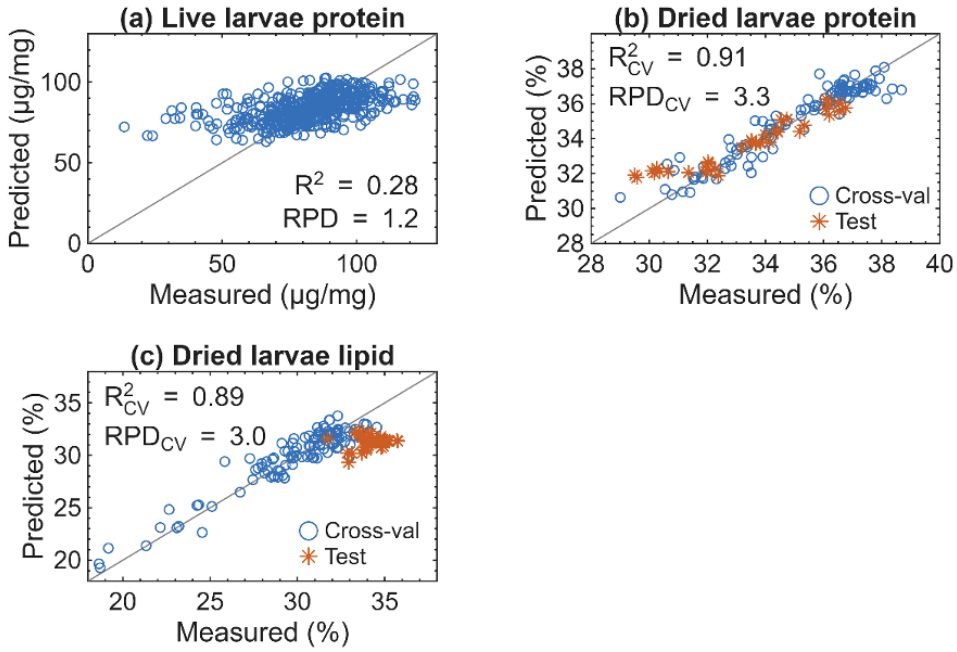

Figure 1. *NIR model predictions for protein in (a) live and (b) dried larvae, and for lipid in (c) dried larvae using the highest-performing models. Predictions from cross-validation of the original training dataset are shown in blue, while predictions from the independent validation dataset are shown in orange.*

Importantly, predictive performance for dried BSFL protein remained consistent when models were applied to an independent validation dataset generated five generations later

(Figure 1b). Predictive accuracy remained high (R^2 = 0.856–0.925), demonstrating strong model robustness and transferability across biologically independent production cycles without recalibration. This result indicates promising applicability for commercial production environments, where biological and environmental variation between production batches is expected.

Prediction of lipid content in dried BSFM also performed well during initial model testing, with models explaining approximately 75–89% of variation in measured lipid composition (R^2 = 0.753–0.887). However, performance declined substantially when assessed using the independent validation dataset, indicating reduced model transferability for lipid prediction across production periods. This suggests additional calibration data spanning broader biological and environmental variation may be required to improve robustness for long-term deployment for the prediction of lipids in BSFL.

BSFL PROTEIN AND AMP CHARACTERISATION

High resolution LC-MS/MS and custom bioinformatic workflows were used to characterise the protein and AMP composition of raw and processed BSFL products.

Industrial processing (commercial drying) reduced the number of detectable proteins from 1,927 in raw (freeze-dried) BSFL to 1,370 in processed BSFL, representing a 28.9% reduction (Table 1). Similar reductions (29.9-32.5%) were observed across Gene Ontology annotation categories, indicating that the reduction in detectable proteins occurred across multiple functional groups. The number of detected immune-associated proteins decreased by 42.7%. The replicate-level analysis confirmed a significant reduction in immune protein richness following processing (paired Wilcoxon signed-rank test, p=0.0079), together with significant reductions in direct immune effector proteins (p=0.01) and pathogen recognition proteins (p=0.01) (Figure 2).

An additional feeding experiment investigated the influence of waste derived feedstocks on BSFL immune-associated proteins and antimicrobial peptide candidates (Figure 3.) Although immune protein richness did not differ significantly among dietary treatments (Kruskal-Wallis, p=0.061), immune protein functional composition differed significantly (PERMANOVA, R^2 = 0.9068, p=0.004) (Figure 3a). This pattern was primarily driven by differences in the number of immune effector proteins, with larvae reared on fruit and vegetable waste (FVW) consistently exhibiting the greatest number.

Antimicrobial peptides are among the most functionally relevant immune effector proteins identified in insects. Using our four tired annotation framework, both canonical AMP families and a broader group of AMP-like molecules were identified. A total of 293, 262 and

254 AMP candidates were identified in larvae reared on FVW, soy okara (SO) and brewers' spent grain (BSG), respectively. Although similar numbers of inferred AMP-like and keyword supported candidates were detected among feedstocks, significant differences were observed in the number of high confidence family assigned AMP candidates (Kruskal-Wallis, p=0.018). Larvae reared on FVW had the greatest diversity (13) followed by SO (11) and BSG (5) (Figure 3b). Differences in AMP family composition were also observed in relation to feedstock, with lysozymes detected across all diets but more abundant in FVW reared larvae, defensins only detected in FVW reared larvae and cecropins detected only on SO reared larvae (Figure 3c).

Table 1. *Summary of the effects of processing (industrial drying) on the detectable BSFL proteome. Protein numbers represent the total number of proteins detected and functionally annotated following LC-MS/MS analysis. Immune-associated proteins were identified using the custom bioinformatic annotation pipeline.*

Protein category	Raw (Freeze-dried)	Processed (Commercially dried)	Reduction (%)
Total proteins detected	1,927	1,370	28.9
GO-annotated proteins	1,731	1,199	30.7
Molecular function annotations	1,468	1,008	31.3
Cellular component annotations	1,379	967	29.9
Biological process annotations	1,109	749	32.5
Immune-associated proteins	**267**	**153**	**42.7**

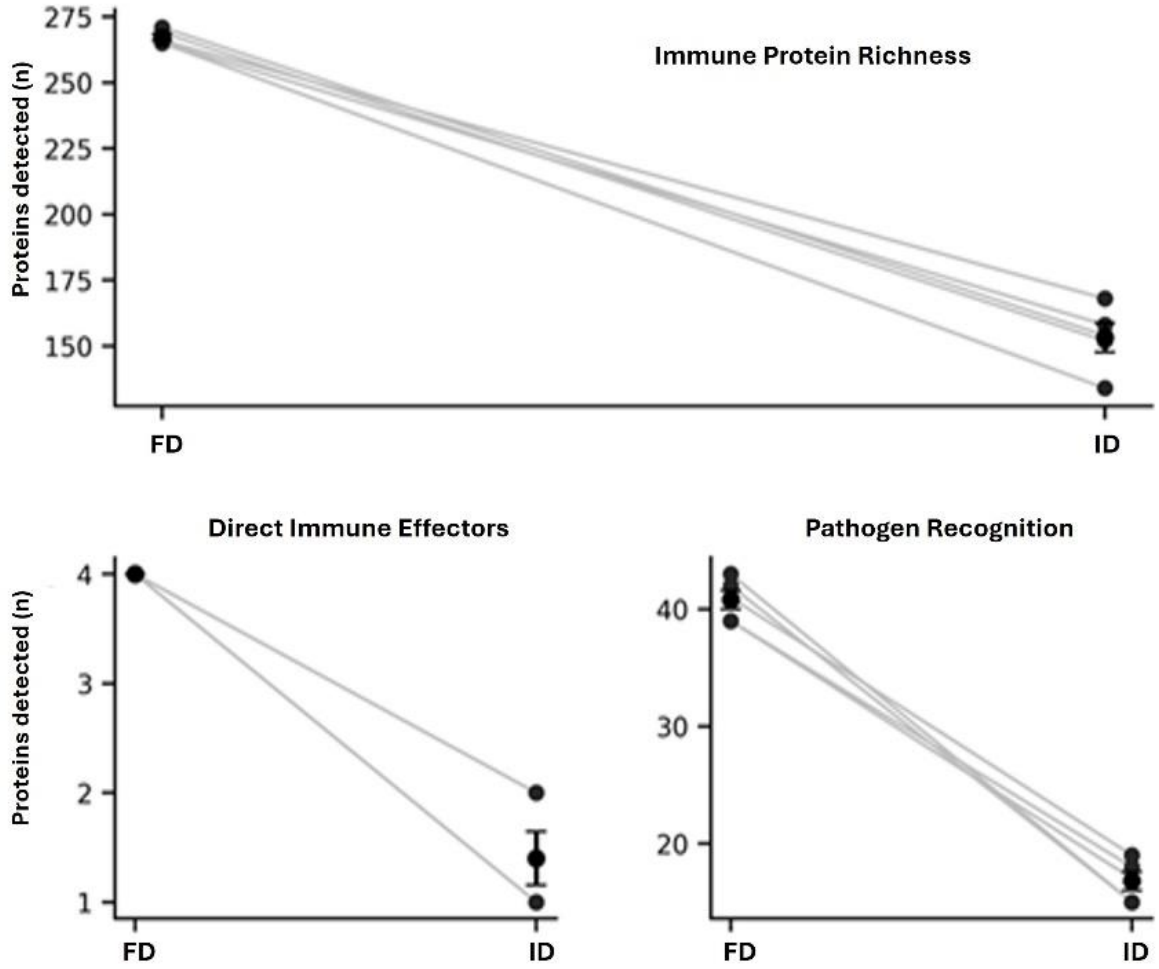

***Figure 2**. (a) Immune protein richness (b) direct immune effector proteins and (c) pathogen recognition proteins from raw (freeze-dried; FD) and processed (industrially dried; ID) BSFL products.*

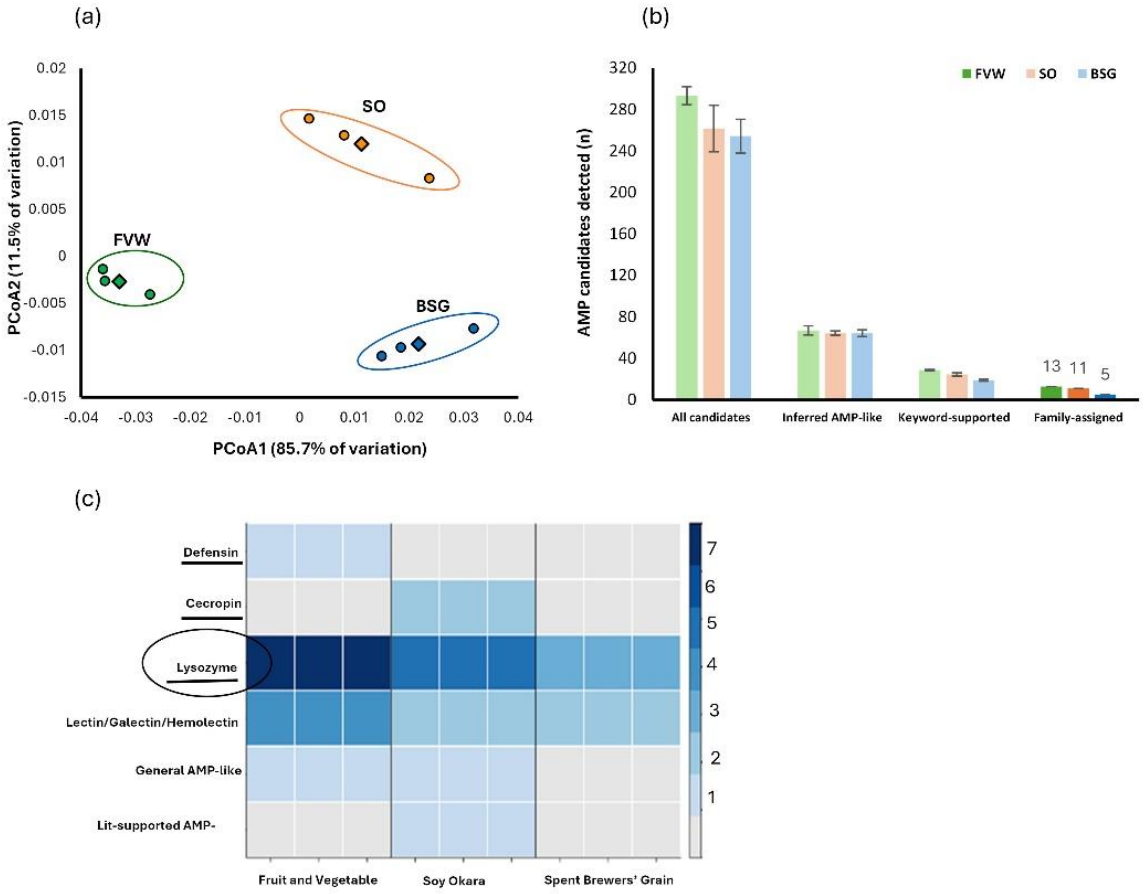

Figure 3. *Influence of waste-derived feedstocks on the immune-associated proteome and antimicrobial peptide (AMP) candidates of black soldier fly larvae (BSFL). (a) Principal coordinates analysis (PCoA) of immune protein functional composition among larvae reared on fruit and vegetable waste (FVW), soy okara (SO) and brewer's spent grain (BSG). (b) Progressive refinement of AMP candidates from all detected candidates to high-confidence family-assigned AMP candidates across feedstocks. (c) Relative representation of recognised AMP families across dietary treatments.*

BSFL INCORPORATED AQUAFEED EFFECT ON PRAWN GROWTH AND SURVIVAL

Dietary inclusion of partially defatted BSFLM influenced growth performance, tissue health and intestinal microbial composition of black tiger prawn.

Growth performance varied among the four dietary treatments (Figure 4). Prawns fed the 50% BSFLM inclusion diet achieved the greatest final body weight and specific growth rate, with both parameters significantly greater than those observed in the 25% inclusion treatment. The control and 75% inclusion diets produced intermediate values. Weight gain and condition factor did not differ significantly among treatments. The hepatosomatic index

was significantly lower in prawns fed the 50% BSFLM diet, while survival was significantly lower in the 75% inclusion treatment.

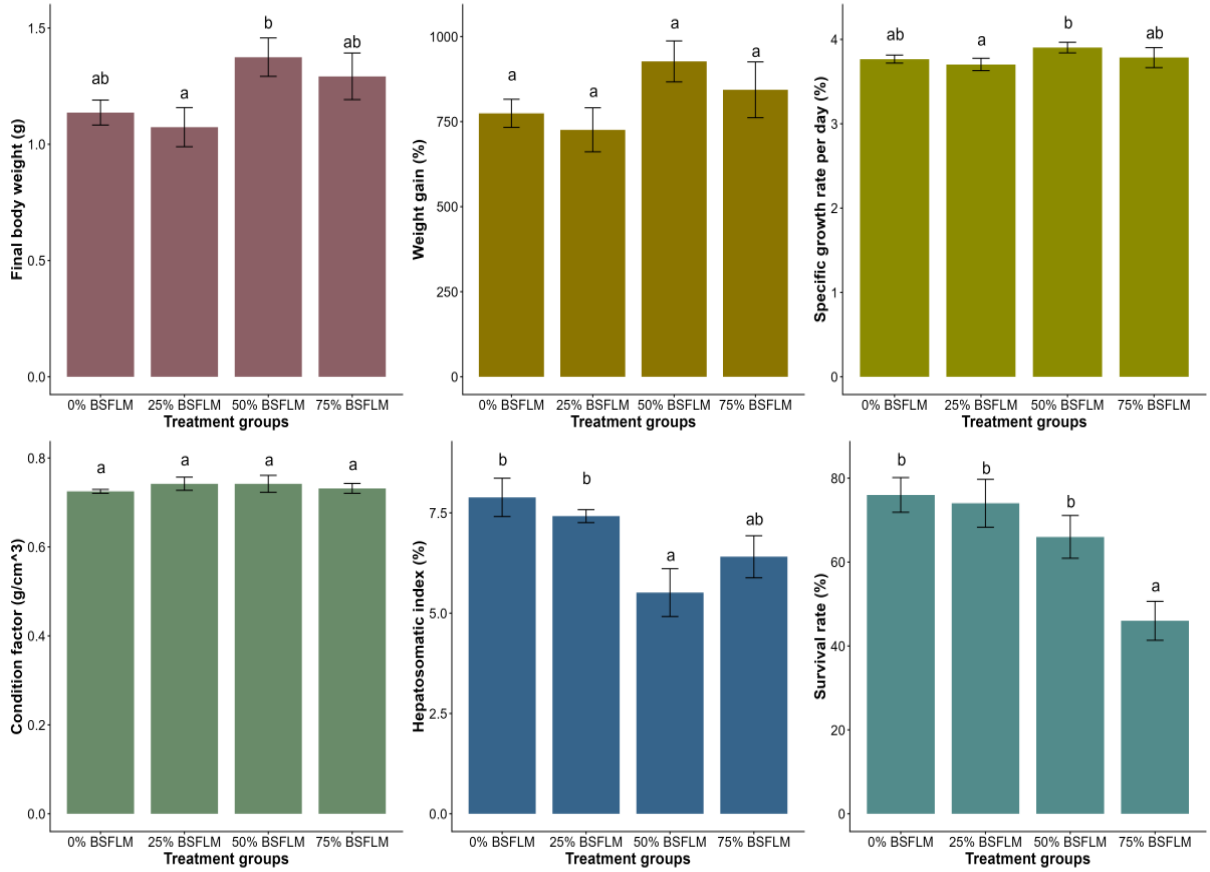

Figure 4. *Six growth performance metrics of Penaeus monodon-fed diets containing different dietary BSFLM levels.*

Histological examination identified no evidence of necrotic enterocytes or cellular damage in the intestine of prawns from any dietary treatment (Figure 5). Mucosal thickness did not differ significantly among the BSFLM-fed groups, while intestinal villus height was greatest in prawns fed the 50% BSFLM diet. Differences in hepatopancreas morphology were also observed among dietary treatments. Prawns fed the 50% BSFLM diet exhibited improved hepatopancreatic morphology, whereas the control and 75% inclusion diets showed greater muscular shrinkage and vacuolisation (Figure 6).

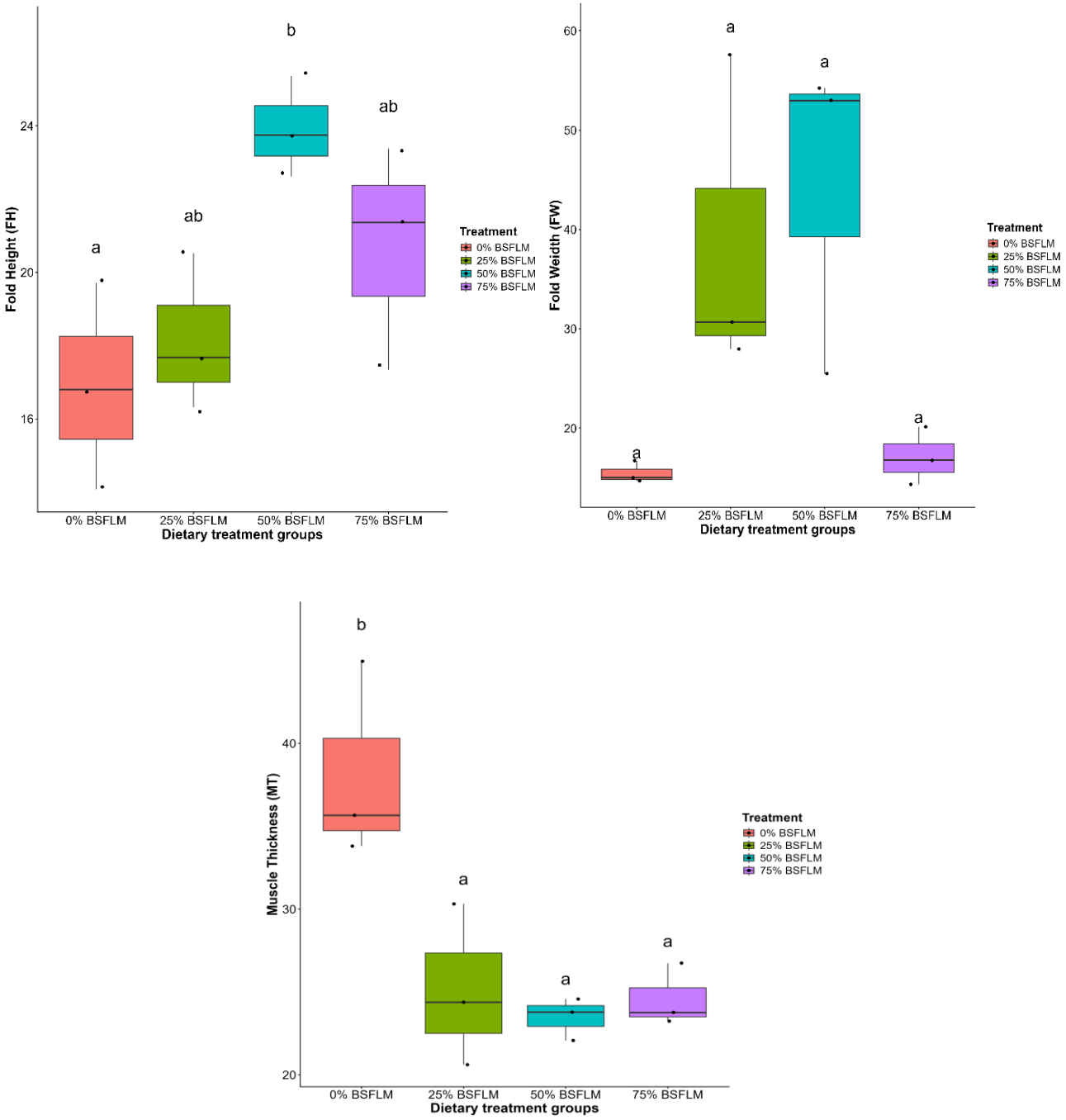

Figure 5. *Effects of dietary BSFLM inclusion on intestinal morphology of Penaeus monodon. FH, FW and MT represent intestinal fold height, fold width and muscle thickness, respectively.*

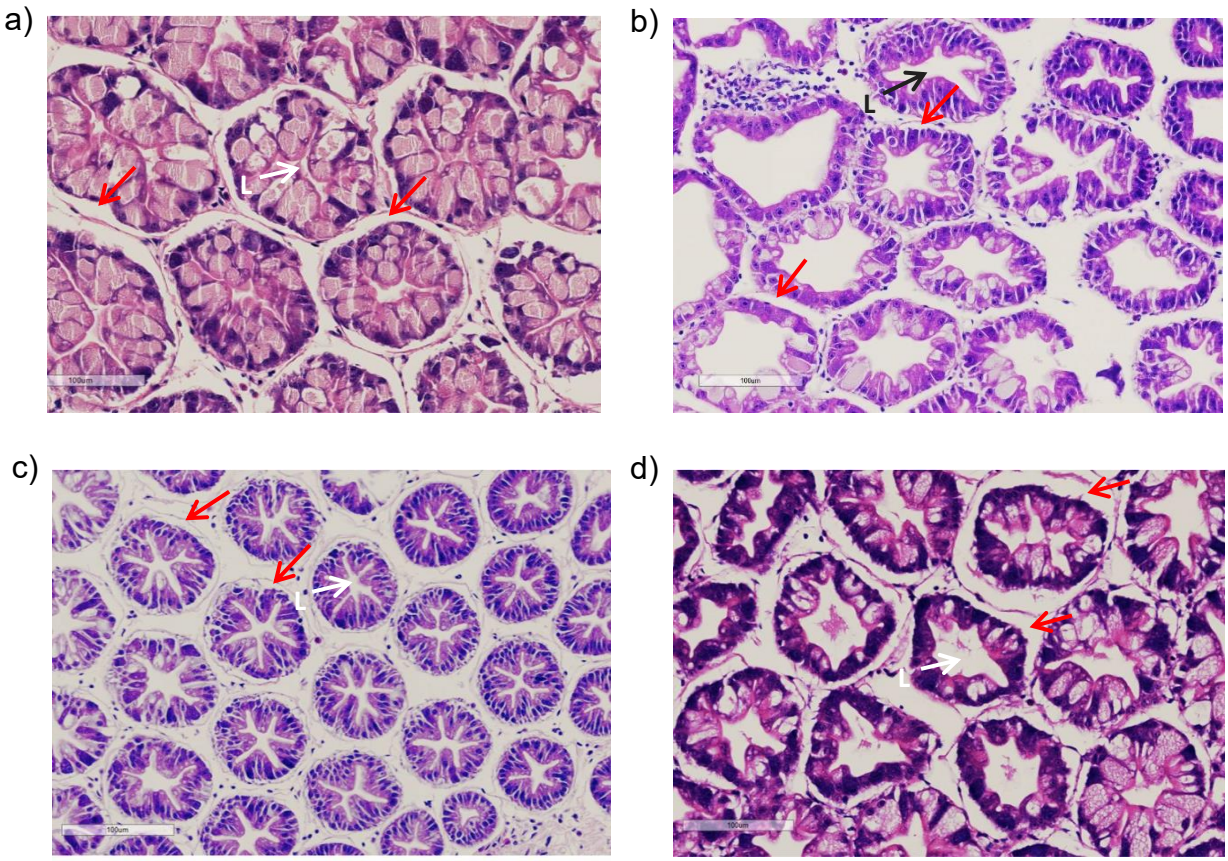

Figure 6. *Hepatopancreas histology of Penaeus monodon fed diets containing (a) 0% BSFLM, (b) 25% BSFLM, (c) 50% BSFLM, and (d) 75% BSFLM. Sections show the organisation and morphology of hepatopancreatic tubules across dietary treatments. L indicates lumen space. Red arrows highlight areas of hepatopancreatic tubule shrinkage observed in treatment groups.*

A total of 1,928,133 high-quality full-length 16S rRNA gene sequence reads were recovered from 20 intestinal samples. Following quality filtering, 850,937 amplicon sequence variants (ASVs) were identified from 17 samples. Alpha diversity did not differ significantly among dietary treatments, with no differences observed in richness (Kruskal-Wallis H = 0.45258, p-value = 0.9292) and evenness (Kruskal-Wallis H = 2.5621, p value = 0.4642)

Despite similar alpha diversity, differences in microbial community composition were observed among dietary treatments (Figure 7). At the phylum level, Proteobacteria, Firmicutes and Bacteroidetes were the dominant bacterial groups. The control diet contained the greatest relative abundance of Proteobacteria, whereas the 50% BSFLM inclusion diet contained the greatest relative abundance of Firmicutes. Increasing dietary BSFLM inclusion was associated with a progressive decrease in the relative abundance of Proteobacteria and Bacteroidetes, and a corresponding increase in Firmicutes. At the genus level, Alkalimarinus, Agarivorans and Vibrio were the most abundant taxa.

total of 1,928,133 high-quality full-length 16S rRNA gene sequence reads were recovered from 20 intestinal samples. Following quality filtering, 850,937 amplicon sequence variants (ASVs) were identified from 17 samples. Dietary treatment influenced the composition of the intestinal microbiome (Figure 6). At the phylum level, Proteobacteria, Firmicutes and Bacteroidetes were the dominant bacterial groups. The control diet contained the greatest relative abundance of Proteobacteria, whereas the 50% BSFLM inclusion diet was characterised by a greater relative abundance of Firmicutes. Increasing dietary BSFLM inclusion was associated with a progressive decrease in the relative abundance of Proteobacteria and Bacteroidetes and a corresponding increase in Firmicutes. At the genus level, Alkalimarinus, Agarivorans and Vibrio were the most abundant taxa. Alpha diversity metrics did not differ significantly among dietary treatments, with no differences observed in Chao richness, Pielou's evenness, Shannon diversity or Simpson diversity (Figure 7).

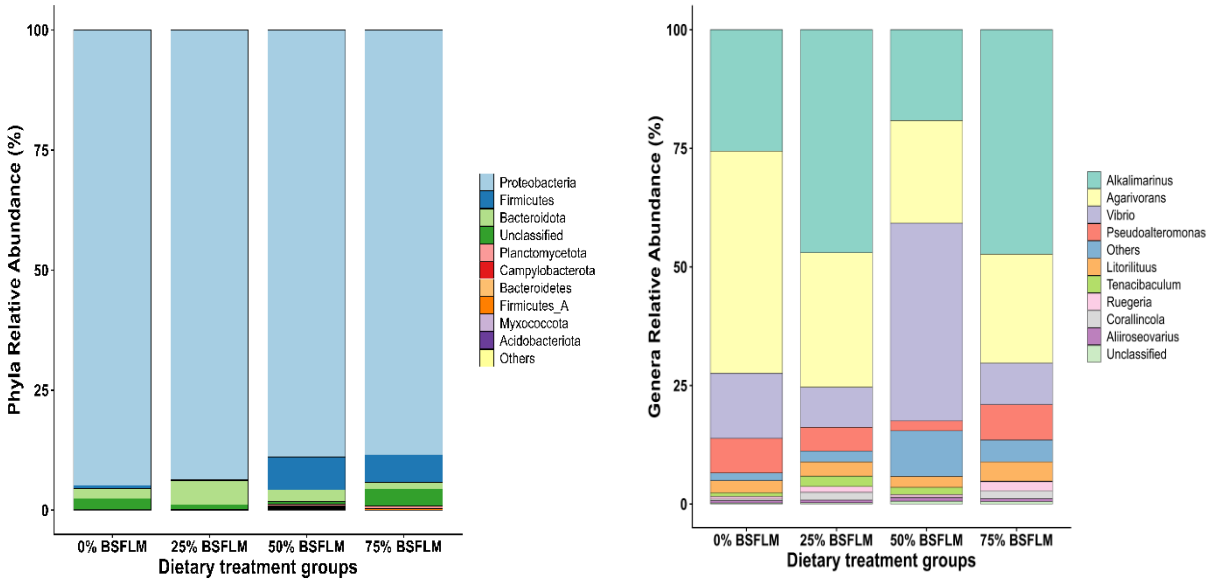

Figure 7. *Effect of dietary BSFLM inclusion on the intestinal microbial community of Penaeus monodon. (a) Relative abundance of the ten most abundant bacterial phyla. (b) Relative abundance of the ten most abundant bacterial genera.*

Principal coordinates analysis (PCoA) based on weighted UniFrac distances demonstrated separation of intestinal microbial communities among dietary treatments (Figure 8A), with the first two principal coordinates explaining 74.15% of total variation. Samples from the 50% and 75% BSFLM inclusion diets were separated from those fed the control and 25% inclusion diets, indicating diet-associated shifts in phylogenetically weighted microbial community composition. PERMANOVA confirmed significant differences in microbial community composition among treatments ($F = 2.108$, $R^2 = 0.327$, $p = 0.0001$, 9999 permutations), while PERMDISP identified significant differences in multivariate dispersion among dietary treatments ($F = 8.967$, $p = 0.0027$; Figure 8B).

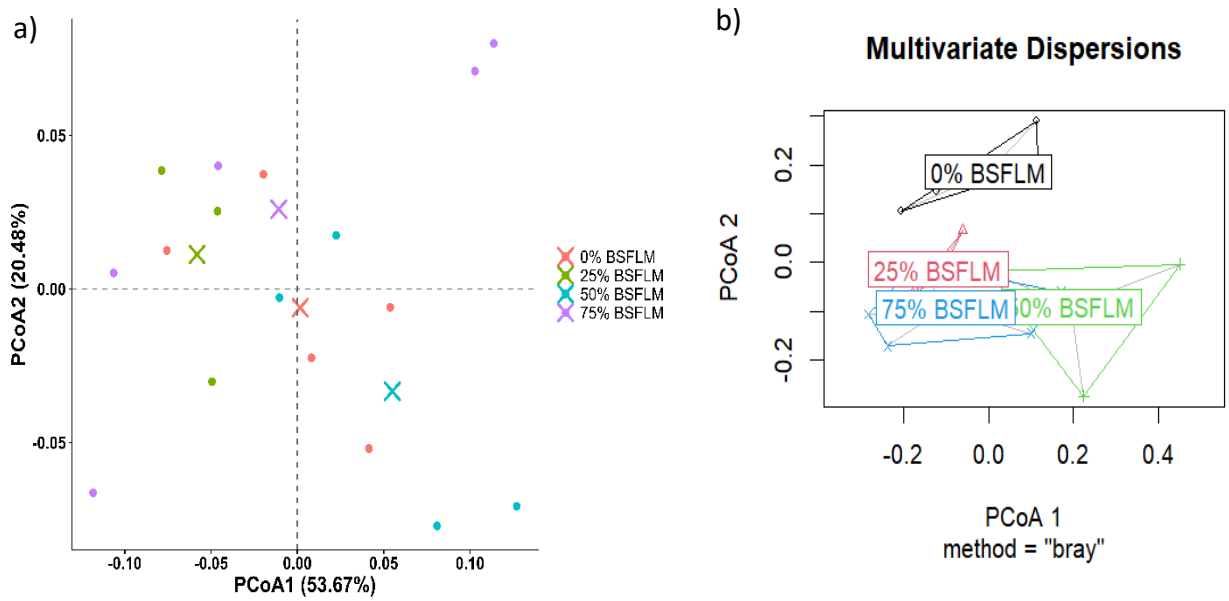

Figure 8. *β-diversity analysis of intestinal microbial communities from Penaeus monodon fed different levels of partially defatted BSFLM. (a) Weighted UniFrac PCoA showing microbial community separation among dietary treatments. (b) PERMDISP analysis showing differences in community dispersion among treatments.*

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

This project addressed key industry limitations relating to BSFL quality assessment, compositional understanding, and application within aquaculture feed systems. The project successfully demonstrated the potential for NIR spectroscopy as a rapid on-farm tool for assessing BSFL nutritional quality, particularly for post-harvest dried products. While predictive performance for live larvae remained limited under the conditions tested, robust prediction of protein content in dried BSFM was achieved and maintained across independent production cycles, demonstrating strong potential for future commercial application.

The project established comprehensive protein and AMP compositional profiles of freeze dried raw and industrially processed BSFL, improving understanding of potential functional compounds present in insect-derived products. Custom proteomic and bioinformatic workflows were developed to enable the systematic identification and classification of immune-associated proteins and AMP candidates, providing a robust analytical framework for future studies. The research demonstrated that the bioactive protein composition is responsive to production variables rather than being a fixed characteristic of the biomass. Collectively, these findings contribute to the growing evidence base supporting BSFL as a potential source of bioactive compounds for animal feed systems and highlight opportunities to optimise the bioactive composition through informed production practices.

Feeding trials demonstrated that partially defatted BSFLM could successfully replace a substantial proportion of conventional protein ingredients in black tiger prawn diets without compromising growth performance. The 50% replacement diet produced the most favourable combination of growth performance, tissue health and intestinal microbiome composition, while higher inclusion levels reduced survival. The observed changes in gut microbial communities indicate that BSFLM inclusion influences intestinal microbial composition in addition to growth performance and tissue health. Together, these findings support the potential for BSFLM as a sustainable aquafeed ingredient and demonstrate the importance of optimising dietary inclusion levels to maximise production performance.

Collectively, project outcomes support the growing role of BSFL within circular and sustainable aquaculture systems while improving industry understanding of quality assessment and feed applications.

RECOMMENDATIONS

- **NIR technologies show strongest immediate application for dried BSFL products**, where prediction performance demonstrated sufficient accuracy to support quality assessment and product standardisation and diversification.
- **BSFL should continue to be supported within circular economy frameworks**, particularly where organic waste streams can be converted into high-value protein ingredients for agriculture and aquaculture. This provides opportunities to improve resource efficiency and reduce reliance on conventional feed inputs.
- **Standardised compositional profiling of BSFL products should be encouraged**, as nutritional and functional variation between products may influence downstream feed performance, end-user confidence, and may open up new product streams.
- **BSFL should continue to be evaluated as an aquafeed ingredient**, both as a replacement for conventional feed proteins and as a functional additive with potential health-related benefits.
- **Further evaluation of BSFLM-induced changes in intestinal microbial communities should be undertaken** to determine their functional significance, including potential impacts on immune function, disease resistance and resilience under commercial production conditions.
- **Future optimisation of BSFL production should consider bioactive composition alongside production efficiency,** recognising that production conditions provide opportunities to develop higher-value functional feed ingredients.
- **Validating the biological activity, stability and bioavailability of identified BSFL proteins and AMPs,** will support their translation into functional feed applications.

NEXT STEPS

The project generated several promising outcomes at varying stages of readiness, requiring different pathways for progression beyond the project period. Future activities should focus on refinement, validation, and translation of project outputs toward practical industry application.

NIR SPECTROSCOPY DEVELOPMENT

- Continued industry support for implementation and field testing of NIR approaches under commercial insect production systems.
- Test and optimise predictive calibration models on deployment-specific NIR hardware, recognising that model performance may vary among instruments.
- Expand calibration datasets across broader feed substrates, production environments, and seasonal conditions to improve long-term predictive robustness.
- Evaluate practical integration of NIR technologies for rapid, lower-cost on-farm quality assurance and product standardisation.

BSFL PROTEIN AND AMP CHARACTERISATION

- Continue AMP discovery and compositional profiling to identify additional candidate bioactive compounds within BSFL products.
- Continue to investigate how feedstock selection, processing and/or other production variables could be used to optimise the bioactive composition of BSFL biomass.
- Undertake functional validation of candidate proteins and AMPs to better understand biological activity and proposed functionality.
- Investigate potential use of identified bio-actives as feed additives for aquaculture and animal production systems.
- Seek continued industry support and partnerships to support progression toward applied translational outcomes.

BSFL INCORPORATED AQUAFEED EFFECT ON PRAWN GROWTH AND SURVIVAL

- Refine understanding of optimal BSFL inclusion levels within aquafeed formulations, recognising BSFL as a complementary ingredient rather than a direct replacement for conventional feed ingredients.
- Investigate whether BSFL-derived bioactive compounds, including antimicrobial peptides identified through Objective 2, may provide functional benefits beyond nutrition alone, such as improved gut health or resilience.

- Investigate whether manipulation of BSFL grow-out diets can be used to produce targeted nutritional or bioactive profiles, with the aim of generating insect-derived feed ingredients tailored for specific aquaculture outcomes.
- Assess transferability of findings to other aquaculture or animal production species where BSFL ingredients or bioactive compounds may provide nutritional or functional benefits.

HIGHER DEGREE BY RESEARCH STUDENTS

JESSICA MADDAMS, PhD CANDIDATE

Supervisors: Prof Andreas Lopata and Prof Kyall Zenger
Project contribution: Objective 2, BSFL protein and AMP characterisation

Jessica led the experimental and analytical components of the project investigating the protein and AMP composition of BSFL. This included designing and conducting the rearing and processing experiments, preparing samples for proteomic analysis, and developing and implementing custom bioinformatic workflows to systematically identify and classify immune-associated proteins and AMP candidates. Jessica analysed the proteomic datasets to characterise the protein and AMP composition of raw and industrially processed BSFL products and determine how waste derived feedstocks influenced bioactive protein composition, generating comprehensive compositional profiles across both production scenarios. Her work delivered key project milestones, established analytical workflows for future studies and contributed to conference presentations and peer-reviewed publication arising from the project.

TALUKDAR JANNAT TAMANNA, PhD CANDIDATE

Supervisors: Dr Mikaeylah Davidson, A/Prof Leo Nankervis and Prof Kyall Zenger
Project contribution: Objective 3, BSFL incorporated aquafeed effect on prawn growth and survival

Jannat led the experimental and analytical components of the project investigating the application of BSFL-incorporated aquafeeds for black tiger prawn production. This included the development and formulation of experimental diets, establishment and management of the feeding trial, dietary treatment administration, animal husbandry, and monitoring of growth and survival performance. Jannat conducted sample collection and contributed to downstream histological and gut microbiome analyses to characterise the effects of BSFL-based diets on prawn health, intestinal microbial communities and production outcomes.

Her work generated critical data demonstrating the potential of BSFLM as a sustainable aquafeed ingredient, identified optimal inclusion levels for prawn production, and contributed to the development of recommendations for future functional feed applications.

PROJECT TEAM

James Cook University: Mikaeylah Davidson, Jessica Maddams, Talukdar Jannat Tamanna, Leo Nankervis, Andreas Lopata, Bronson Philippa and Kyall Zenger

FlyFarm: Constant Tedder, Quyen Banh and Tom Shatalau

Tassal: Aaron Wingrove, Courtney Remilton and Tim Shepherd

PUBLICATIONS LIST

Maddams, J.C., Lopata, A.L., and Zenger K.R., (date TBC). Waste-derived feedstocks and industrial drying influence antioxidant capacity and immune-associated protein composition in black soldier fly larvae (*Hermetia illucens*). Journal of Insects as Food and Feed. *In review.*

Davidson, M.J, Philippa, B., Tedder, C., Jerry, D.R., White, R., Zenger K.R., (date TBC). Near-Infrared Spectroscopy for Rapid On-farm Protein and Lipid Assessment in Black Soldier Fly Larvae. *In preparation.*

Talukdar JT, Davidson, M.J, Das, S., Zenger K.R., Nankervis, L. (date TBC), Dietary inclusion of partially defatted black soldier fly larvae meal modulates growth performance, tissue health and intestinal microbiota of black tiger prawn (Penaeus monodon). *In preparation.*

Conferenced papers:
Davidson, M.J, Philippa, B., Tedder, C., Banh, Q., Jerry, D.R., White, R., Zenger K.R., (2026). Near-Infrared Spectroscopy for Rapid On-farm Protein and Lipid Assessment in Black Soldier Fly Larvae. Insects to Feed the World Conference, June 2026, Turin, Italy.

Maddams, J.C., Zenger K.R., and Lopata, A.L., (2026). Modulating Bioactive Compounds in Black Soldier Fly Larvae Through Waste Feedstocks and Industrial Processing. Insects to Feed the World Conference, June 2026, Turin, Italy.

ACRONYMS & ABBREVIATIONS

BSF	Black solider fly
BSFL	Black solider fly larvae
BSFLM	Black solider fly larvae meal
NIRs	Near Infra-Red spectroscopy
AMP	Antimicrobial peptide

Closing the circular food economy: advanced technologies to optimise black soldier fly larvae nutritional composition for inclusion in aquafeed

Mikaeylah Davidson, Jessica Maddams, Talukdar Jannat Tamanna, Leo Nankervis, Andreas Lopata, Bronson Philippa & Kyall Zenger

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