METABOLOMICS ON BLOW FLY LARVAE: PMI ESTIMATION

Z.K. Perveen, M.E. Benbow, A. Bonicelli, N. Procopio

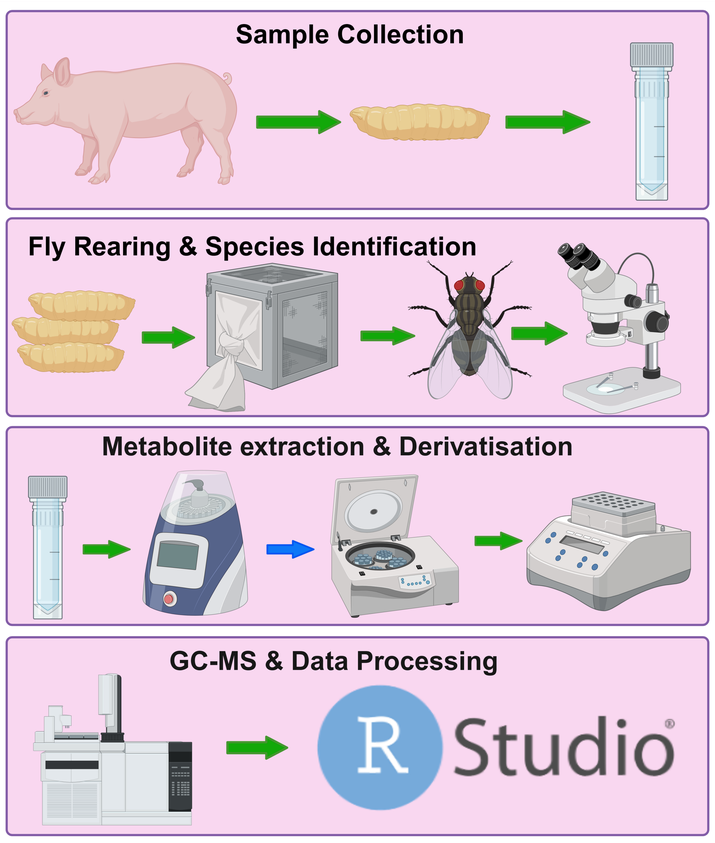
School of Law and Policing, University of Central Lancashire

INTRODUCTION

Accurate estimation of post-mortem interval (PMI) is crucial for forensic investigations. Traditional method such as decomposition assessment and entomological techniques, can be influences by environmental factors, leading to variability in PMI estimation. Metabolomics, a branch of -OMICS, offers a novel approach and promising approach by analysing biochemical changes occurring in blow fly larvae as they develop on decomposing biological material.

This study integrated metabolomics with forensic entomology to enhance the accuracy and reliability of PMI estimation contributing to both forensic science and broader ecological sciences.

METHODS



RESULTS

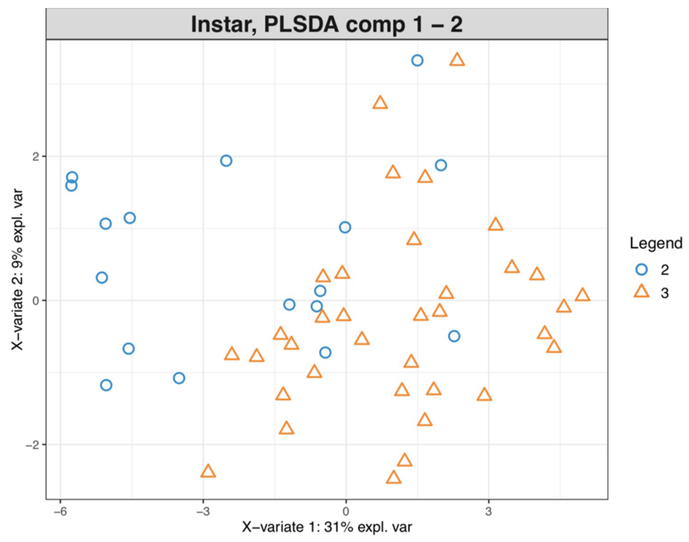


FIGURE 1. PLS-DA model of larval metabolic profiles and their developmental stage.

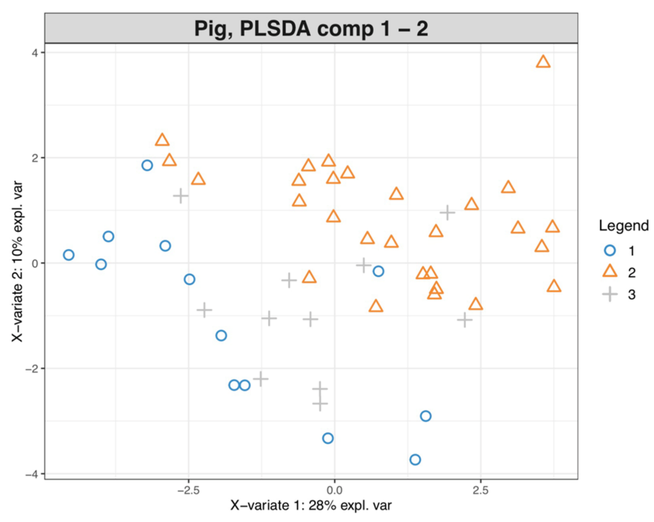


FIGURE 2. PLS-DA model illustrating the metabolic profiles of larvae in relation to the specific pig carcass from which they were collected.

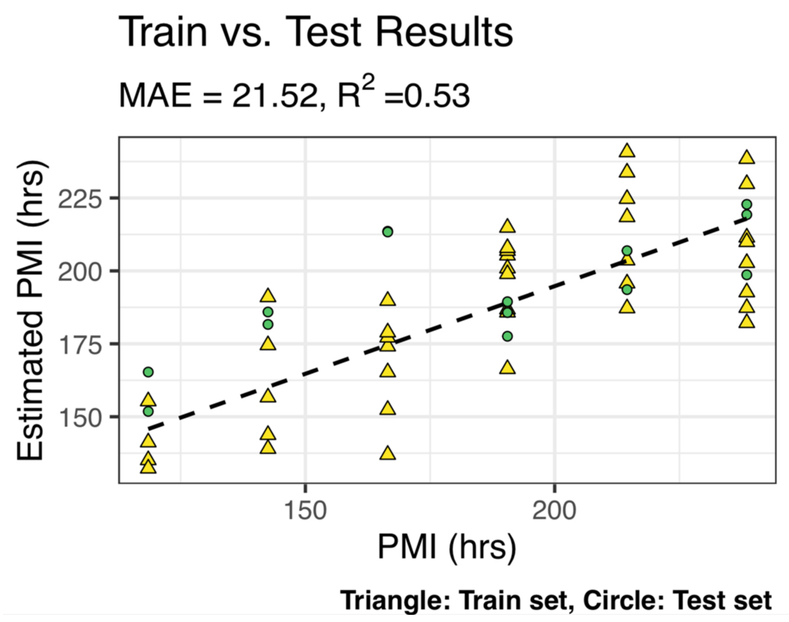
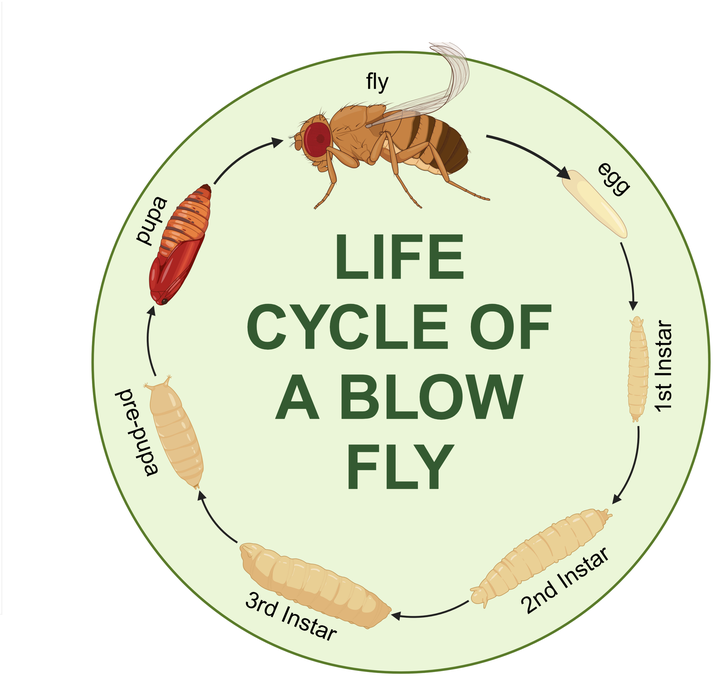


FIGURE 3. Predictive-model (partial least squared regression) for PMI estimation using metabolic profiles of blow fly larvae.



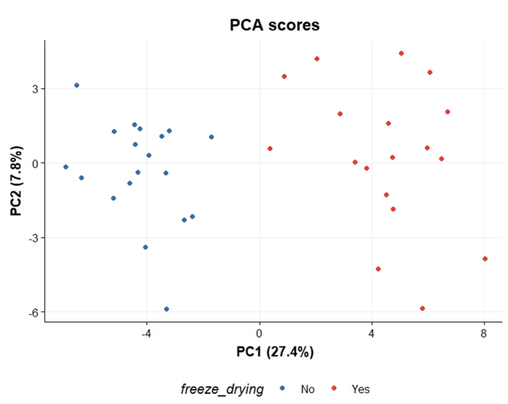


FIGURE 4. PCA score of freeze dried and non-freeze dried samples.

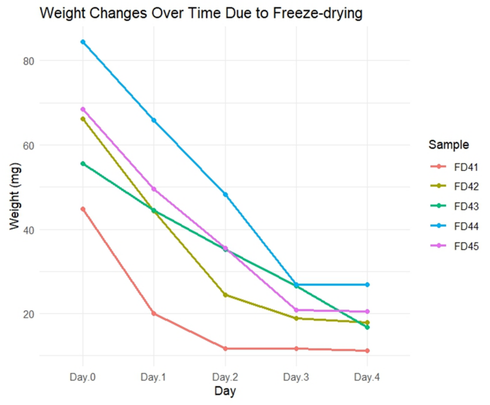


FIGURE 5. Line graph of larval percentage weight loss over 96 hours.

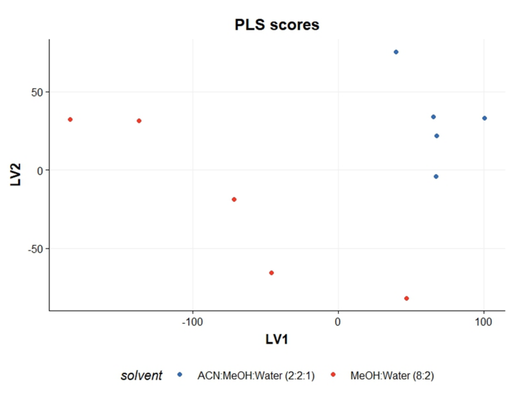


FIGURE 6. PCA scores of samples were extracted using the solvent systems MeOH:H_2O (4:1) and ACN:MeOH:H_2O (2:2:1).

DISCUSSION

KEY FINDINGS

1. **Metabolic variability across instars (FIGURE 1.):** metabolic profiles of larvae significantly varied between developmental stages.
2. **Inter-corpse variability:** Maggots collected from different pig carcass displayed distinct metabolic profiles, reflecting differences in decomposition and biological variability (FIGURE. 2.)
3. **Predictive-modelling:** A partial least squares regression model was developed. The model demonstrated mean absolute error of 21.52 hours for PMI estimation at around 200 hours (FIGURE 3), laying the groundwork for further refinement.
4. **Method optimisation:** Freeze-drying (FIGURE 4.) for 72 hours (FIGURE 5.) and Acetonitrile:Methanol:Water (FIGURE 5.) proved to be the most effective treatment and solvent for metabolite extraction.

LIMITATIONS

- **Environmental variability:** differences in temperature and decomposition rates affected larval development, which would influence metabolic profiles.
- **Sampling Constraints:** a minimum of 25mg of samples is needed
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- **Model refinement:** while promising, the predictive model requires further validation, with larger datasets and additional controlled experiments to improve accuracy.
- **Inter-species differences:** further research is needed to explore species-specific metabolic variations.

CONCLUSION & FUTURE DIRECTION

This study demonstrates the feasibility of using metabolomics as a complementary tool for PMI estimation in forensic entomology. The findings highlight the potential of metabolic profiling in identifying larval developmental stages and refining PMI estimation models. Optimisation of sample preparation methods, particularly freeze-drying and solvent selection, has enhanced metabolite recovery, contributing to more robust analytical workflows. Future research will focus on refining predictive models and exploring species specific metabolite markers to further strengthen forensic applications.

ACKNOWLEDGEMENTS

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