Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice
Czech Republic

Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Kifayatullah, M.Sc.	**First name(s), surname, titles of supervisor:** Hamid Niksirat Hashjin, M.Sc. Ph.D.
Title of PhD thesis: The cellular and molecular characteristics of hemolymph in crayfish	

REVIEWER:

Surname: Khor	**Institution**: Universiti Malaysia Terengganu, Terengganu, Malaysia
Name: Wai Ho	
Titles: Dr.	**E-mail**: waihokhor@gmail.com; waiho@umt.edu.my
Please describe your professional relationship to the PhD student: Thesis reviewer	**Please describe your field of expertise:** crustacean physiology, aquaculture biotechnology

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field **(extent ¼ – ½ page)**:

Knowledge on the clotting system and mechanisms of decapod crustaceans are essential and useful for various fields, including disease management and diagnosis, aquaculture and fisheries sectors, biomedical applications, and ecosystem functioning. The thesis aims to contribute to this by the detailed characterization of hemocytes during coagulation and phagocytosis, and further comparison of proteomic profiles of clot and non-clot hemolymph of marbled crayfish using advanced techniques, i.e., transmission electron microscopy in the former, and high-resolution mass spectrometry in the latter. The experimental design and methods used are suitable and represents the latest available technique in the field, and the candidate successfully and appropriately deduced the results from the employed techniques, thereby advancing the current field of crustacean hemocyte morphological changes, the mechanisms during clotting process, and proteins that are potentially involved. The review and the discussion sections in chapter 3 & 4 are built upon key and latest publications in the field of clotting in decapod crustaceans, and the findings of the PhD thesis are useful in the international context, especially in maintaining the health and welfare of economically crustaceans in captivity during aquaculture. Additionally, the identified proteins that were significantly expressed during clotting of marbled crayfish could also have implications in biomedical applications. In conclusion, the PhD thesis demonstrates a high level of competitiveness in the international context and presents innovative research findings that address the knowledge gap in the field of crustacean hemolymph and clotting mechanisms.

Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicality of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis **(extent ¼ – ½ page)**:

In general, the PhD thesis framework is sound and valid, and the thesis showcases strength in experimental design and alignment with existing literature. The main texts of most chapters are succinct and clear, and I enjoyed reading through the whole manuscript. The arrangement of chapters is in logical order – Chapter 1 introduces the main ideas that were the main focuses of the PhD thesis; Chapter 2 provides in-depth explanation and dissection of what has been known on the hemolymph of crustaceans, and the effects of various environmental factors on the innate immune system of crustaceans. The knowledge gaps highlighted in this chapter serves as the research problems for the subsequent two chapters; Chapter 3 describes, in detail, the ultrastructural changes of hemocytes during coagulation and phagocytosis after injury via the use of TEM, and reveals interesting and novel discoveries on the changes in morphology of the cytoplasmic granules, and the phagocytosis process of hemocytes; Chapter 4 dives into the molecular characterisation of the clotting mechanisms by comparing the proteomic profiles of clot and normal hemolymph, with four significantly regulated proteins identified; Chapter 5 concludes the thesis with a general discussion, merging the findings of this study. The published articles are in line with the proposed objectives. The findings of the thesis are novel and contribute significantly to the understanding of clotting mechanisms of crustaceans. However, below are some comments/concerns that I think would further improve the overall clarity structure of the thesis:

1. The title 'The cellular and molecular characteristics of hemolymph in crayfish' does not reflect the three objectives. Please consider revising it.
2. The current General discussion section seems more like a summary of each chapter, especially that of chapter 2 and 3, rather than a thorough interpretation of findings at a larger context, from the aspect of theoretical, practical, and methodological implications. Mr. Kifayatullah managed to compare and contrast his findings with the existing literature, and discuss the discrepancies in theories revolving the clotting machanisms of crustaceans. However, some acknowledgement of the limitations or constraints of the research, or any potential sources of bias or weaknesses that could have affected the validity of the findings, and avenues for future research to address these limitations or the incorporation of new technologies, could be added in this chapter as well.

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OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

Formatting

The overall formatting of the thesis follows that has been outlined in the guideline for writing the Ph.D. thesis at the FFPW USB. The length of the Ph.D. thesis is within the acceptable range of 60-120 pages. However, below are some noticeable errors that require further revision:

1. Page 11, 80: please italicise 'Penaeus monodon'
2. Page 55: please italicise 'in vitro'
3. Page 56: do you mean 'plasma clotting protein' instead of 'plasm clotting protein'?
4. Page 62, 63: There are 'supplementary Table S3', and 'supplementary Table 1'. Two differing formatting for supplementary tables. Please choose only one.
5. Page 81: please italicise 'in situ'
6. Page 87: 'Biological Reviews' abbreviate to 'Biol. Rev.'
7. Page 93: In the first publication listed, author (Mengal, K.) is not italicised, and this publication lacks publication year, volume, and page number.
8. Page 93: Publication lacks page number – Effects of environmental factors on the cellular and molecular parameters of the immune system in decapods. Details can be found in https://www.sciencedirect.com/science/article/abs/pii/S1095643322001908
9. Page 93: Publication lacks page number, the whole sentence should be sentence case, and species name not italicised – Nephroprotective Effect of Cinnamon cassia and Azadirachta indica on Titanium Dioxide Nanoparticles.

Sentence Structure & additional comments

10. Page 6: Please rephrase "Crayfish have been used to investigate population dynamics, habitat preferences, and the effects of environmental stressors on aquatic communities in the field of ecology (Reynolds et al., 2013).". I think what you are trying to convey is that the ecological aspects of crayfish, including their population dynamics, habitat preferences, and the effects of environmental stressors were also extensively investigated.
11. Page 6: Please revise "plays critical roles in their physiology and immune system". Do you mean 'plays critical roles in the maintenance of their physiology and immune systems."?
12. Page 8: "The molecular component of crustacean's innate immunity comprises on diverse array of peptides and proteins, such as lectins, antimicrobial peptides, clotting factors, and complement-like proteins.", could be revised to "The molecular components of crustaceans' innate immunity comprise a diverse array of peptides and proteins, such as lectins, antimicrobial peptides, clotting factors, and complement-like proteins". However, are peptides and proteins a part of the 'molecular' component?
13. Page 8: "pathogens ~~agents~~"
14. Page 8: Do you mean "Crustaceans are 'constantly' exposed to"
15. Page 8: "Additionally ~~However~~, it was observed that", and change 'animals' to 'crustaceans' to avoid confusion.
16. Page 8: Suggest each environmental stressor be in its own paragraph, or if not, combine that of temperature and the other stressors.
17. Page 10: Please combine these two sentences – "The phagocytic cells recognize and ingest invading pathogens and apoptotic cells, followed by their degradation and elimination (Flannagan et al., 2012). The process of phagocytosis, carried out by specialized cells called hemocytes in invertebrates, involves the engulfment of foreign entities (Giulianini et al., 2007).". Are phagocytic cells equivalent to hemocytes? The two sentences essentially convey the same message, but with

slightly different information. Hence, please revise and combine them.

18. Page 10: is it “Charles A. Janeway” or “Janeway”?
19. Page 11: “based on ‘THE’ morphological characteristics” of? Or simply remove ‘based on morphological characteristics’.
20. Page 12: Instead of stating that “Hemocyte granules have been described by several investigators”, I would suggest changing the sentence to “…described in several invertebrates”, as the author highlighted hemocyte granules of few species instead of focusing on the investigators.
21. Page 56: “The parthenogenic marbled crayfish…. human cancer research.”, forming of a paragraph with just this sentence is insufficient. Try to add more introduction as to why P. virginalis is a suitable model for hemolymph coagulation study in crustaceans.
22. Page 56: “Therefore, this study….animal”, suggest to revise to “Therefore, this study applied ….. to identify proteins, especially those with potential roles in coagulation and immunity, in the HEMOLYMPH proteomic profiles of P. virginalis.”
23. Page 57: Section 2.1, what was the sex of the six individuals? Will there be a sex-specific proteomic difference in the hemolymph of P. virginalis?
24. Page 57: stored at -80C until subsequent analysis.
25. Page 60: In figure 2D, there is no error bar for control?
26. Page 61: In the figure legend, should it be number of genes or number of proteins?
27. Page 63: Consider revising ‘statistically significant differentially abundant proteins’ to ‘differentially abundant proteins’. The term ‘differentially’ already implies ‘statistically significant’.
28. Page 64: The postulate that the activation of apoptotic pathways in hemocytes may lead to the cleavage and degradation of C- type lectins or their associated signaling molecules is a little far fetch. Are there any literatures that link hemocyte’s apoptotic pathway activation with the degradation of signaling molecules? C-type lectins trigger signaling pathways to activate immune responses to invading pathogens (Geijtenbeek & Grnghuis, 2019; doi: 10.1038/nri2569), and its injection induced hemocyte response in shrimp (Li et al., 2022; doi: 10.1016/j.fsi.2022.04.017). But how would the activation of apoptotic pathways in hemocytes lead to the degradation of C-type lectins?
29. Page 66: Please add citations to support the statement of “Considering the immune-related roles of the reverse transcriptase domain-containing protein”.
30. Page 71: The sentences ‘clotting proteins are pre-synthesized and stored in the granules of hemocytes and plasma to enable a rapid response to wounds’ and ‘are likely pre-synthesized and stored, enabling a swift response to injury in the form of coagulation’ are repetitive in the Conclusion section.
31. Page 80: “immune parameters and the abiotic stress mechanisms of ~~the~~ commercial decapod species”.
32. Page 80: The first paragraph under the subsection of “Effects of environmental factors on the cellular and molecular parameters of the immune system in decapods” can be omitted. This paragraph deals with a notion that is still unsure, and I think just a brief description of the three types of hemocytes found in crustaceans is sufficient.
33. Page 80: “suggesting that only these two types of hemocytes are capable of it” can be removed, it is redundant with the first half of the sentence.

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FINAL RECOMMENDATION

☒ **PhD thesis can be recommended for defence**
☐ **PhD thesis can be recommended for defence with reservations**
☐ **PhD thesis cannot be recommended for defence**

6 June 2023, Kuala Terengganu, Malaysia
……
Date and place

Khor Wai Ho
……
Name and signature