



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Guo Wei, M.Sc.	First name(s), surname, titles of supervisor: Dipl.-Ing. Antonín Kouba, Ph.D.
Title of PhD thesis: Burrowing behaviour in crayfish	
REVIEWER:	
Surname: Horwitz	Institution: Centre for Ecosystem Management, Edith Cowan University, Australia
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Titles: Prof.	
Please describe your professional relationship to the PhD student: None	Please describe your field of expertise: Environmental Sciences

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**):

The Candidate has produced a compendium of research that is bound by the commonalities of invasive freshwater crayfish and burrowing behaviour. It builds on research that has been undertaken by the candidate's colleagues. Individual research components investigate important topics (reproductive effort during periods of drought, impacts of pharmaceutically active compounds and herbicides in aquatic systems, and competitive interactions between (over)invasive crayfish species), and the potential was there for a genuinely original PhD thesis to bring these together in a unique synthesis around the degree to which freshwater crayfish are wedded to burrows. Instead the candidate deals with them individually so to my mind there is no real *thesis*. The three publications are very good in quality; they draw heavily on previously published work so there is no real development of methodology, with the possible exception of burrow morphometry. The finding of atypical burrowing behaviour following PhAC exposure is an original outcome of the candidate's work. In terms of competitiveness of the PhD, I regard this to be in the lower half of the 50 PhD theses I have supervised or examined (mainly from Australia).



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, logicity 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**):

Structural factors of the thesis are:

- I. four invasive species were investigated (Marbled crayfish for chapters 2,5,6; red swamp crayfish for chapters 3,4; calico crayfish in Chapter 5 and spiny-cheek crayfish in chapter 6);
- II. three experimental laboratory set-ups (post-embryonic development chapter 2; toxicology for chapters 3 and 4; and interspecific agonistic behaviour for chapters 5 and 6);
- III. 3 published papers (two of them with the candidate as senior author) and 3 unpublished manuscripts;
- IV. Four goals of the research are sought: a) literature review; b) drought conditions and reproduction; c) burrowing behaviour and water contamination; and d) interspecies agonistic behaviour.

Overall the four goals were individually achieved.

The publications are in two very high quality journals (Freshwater Biology, and Science of the Total Environment) and one of moderately high quality journal Aquatic Ecology).

For an international PhD thesis I would have expected conceptual development to bring these four goals together into one cohesive model (in the first chapter), and then to evaluate the model in the final chapter.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Chapter 1 outlines the scope of the study. The rationale of the scope is based on elements of burrowing behaviour for which there are gaps but the rationale for how these gaps are related to one another is weak. Four goals are stated and in their own right they are meaningful and relevant. The challenge for the candidate is how to make them all related to one another – how to make a coherent story from them about burrowing behaviour. For example, how are reproduction, toxicology and competitive behaviour linked to burrowing behaviour – what is the conceptual model? In the absence of such integration the literature review is not publishable.

The section on measuring the three dimensional structure of burrows was very interesting and showed originality.

The reference list is extensive and generally comprehensive. The formatting of the references was excellent for journal articles. For non-journal articles publication details (editor, published by, place etc.) were missing/incomplete.

Chapter 2 is published in the journal *Freshwater Biology*, and the candidate's contribution was 40% from a paper with 7 co-authors. It is a comprehensive experimental study. The paper is an important piece of work regarding post-embryonic requirements for burrow water for freshwater



crayfish (in this instance the marbled crayfish). There was a problem with the control group and the authors sought comparison with 'common observation' – can this be verified in the literature?

Chapter 3 is published in the journal *Science of the Total Environment*. It describes an careful experimental exposure of the red swamp crayfish to environmental concentrations of pharmaceutically active compounds. Detecting significant and some differences that are non-significant, for burrow size of females, the authors draw conclusions that exposure to such compounds, particularly in the context of projected changes to climate with increased drought conditions, are likely to diminish reproductive capacity for burrowing crayfish, and even more so for sensitive species.

In crayfish species elsewhere in the world burrow volume and depth is a function of groundwater fluctuations, the period of time a crayfish has spent in the burrow, and the size of the crayfish. Arguably these are all controlled in this experiment, although there were no measures of water level in the experimental burrows, so a question arises as to whether the burrow size and depth was related to the water level (and its fluctuations) and humidity levels in each container – why weren't these included in the experimental measures?

I also was wondering what were the physico-chemical conditions in the water or the burrow, and whether a) that was homogeneous across all containers, and b) whether there might have been any effect of the conditions in the burrow and the active substances to enhance or degrade them.

Chapter 4 is an unpublished manuscript, with the candidate as the senior author and there being another 10 co-authors. It details a treatment of the burrowing extent of red swamp crayfish under exposure to two herbicides. The chapter is very similar to the previous one, repeating method, and even phrases and sentences. No mention of acclimation is present in the chapter; crayfish were collected and were put directly into experimental conditions for 28 days. There is also no discussion of the possibility that all crayfish were already exposed to herbicides (given they were all sourced from an apparently urban source – Erb in Hungary).

Chapter 5 is published in the journal *Aquatic Ecology*, and the PhD candidate is second named author (from a list of 6 authors) with a contribution of about 25% towards the overall paper. In this chapter the authors seek to establish a case for marble crayfish being able to exclude calico crayfish from habitats due to their agonistic behaviours, and to test whether this might be able to be demonstrated experimentally for shelters/refuges in a laboratory set up.

Chapter 6 is an unpublished manuscript. Like Chapter 5, the experimental design tests the effects of shelter on the interspecific interaction, but only between sexually undifferentiated marble crayfish and male or female spiny cheek crayfish. But these are not controlled - this could have been achieved by testing the intraspecific male x female, male x male and female x female for the spiny cheek crayfish, and marbled crayfish x marbled crayfish. Such tests would have established a variance around the likelihood of dominance *per se* for these two species, rather than attribute dominance and shelter exclusion to the presence of marbled crayfish.

More substantive tests of morphometry (ie. not just weight but also cheliped size and carapace width) as determinants are warranted. There is some evidence to suggest that the size of the



propodus and dactyl of the cheliped is important in agonistic encounters (Gherardi et al. 2000) and that it is therefore a better predictor of dominance in freshwater crayfish (than weight).

In the Discussion there are several paragraphs that are duplicated, and while they are good paragraphs (with the exception of one sentence at the end of paragraph 3 on page 95 which is uninterpretable), this makes it difficult to comment further on this chapter.

The final chapter presents a synthesis of each of the three areas of investigation. For Chapter 7, the challenge for the thesis was to bring the four species and the three investigations together under the umbrella of 'burrowing behaviour'. The synthesis does this partially only, pointing to broader issues of climate change and biological invasions.

Questions

1. How might post-embryonic development, exposures to environmental concentrations of PhAC and herbicides, and agonistic behaviours of interspecies interactions, influence one another in terms of burrowing behaviour?
2. The red swamp crayfish collected from Erb in Hungary may have been exposed to herbicides already. Given that no acclimation was used, could this have influenced the lack of differences between control and treatment exposures?

FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence**
 PhD Thesis can be recommended with reservations for defence
 PhD Thesis can not be recommended for defence

Date and place: 5th July 2020, Fremantle, Western Australia

Name and signature: Professor Pierre Horwitz