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### Review of USB FFPW PhD Thesis

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<b>Title of PhD thesis:</b> Burrowing behaviour in crayfish	
<b>REVIEWER:</b>	
<b>Surname:</b> Tricarico	<b>Institution:</b> Department of Biology, University of Florence, Italy
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<b>Please describe your professional relationship to the PhD student:</b> I hosted him for a research stay in Florence.	<b>Please describe your field of expertise:</b> Biological invasions, behavioural ecology of crayfish, management of crayfish and other aquatic alien species

## 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**):

Burrowing behaviour in crayfish is a particularly important topic to be investigated because burrows allow the individuals to overcome critical moments of their life cycle, and in invasive species it can favour their successful establishment and invasiveness. Despite its importance, there are still many knowledge gaps about this behaviour, considering that it can be affected by a number of variables and that can change among species and within the same species between the native and invaded area. The thesis addresses some of these gaps and also considers the effects of pollutants and competition between different NICS on this behaviour. The results are noteworthy and important, improving the knowledge on crayfish biology, particularly of invasive crayfish present in Europe. The level and quality of the thesis are very good, as also testified by the already published articles in high impact factor journals. The work is thus very interesting and timely for the scientific pertinent field, and is relevant for pure and applied research: the effect of pollutants on behaviour is indeed becoming an important research area of applied ethology not only for terrestrial species but also for aquatic ones. Even if the candidate analyses the behaviour of only NICS, the results of the present thesis are relevant to understand the potential consequence of pollution on native crayfish, the dynamics between different invasive crayfish and the consequence on management of NICS. Finally, marbled and calico crayfish are emerging



as the new invaders in Europe and studies on these species are necessary to understand their potential relationship in the wild.

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***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**):

The thesis has four main aims, reported at the end of Chapter 1, for whose achievement the candidate conducted several laboratory experiments. Overall, the thesis is a good and solid work, well and clearly structured, and the English is fluent and well written, even if some corrections are suggested in the specific box below. The methods are appropriate, clearly illustrated and explained. The statistical analyses have been conducted in the appropriate way. The results are well presented, discussed with a good critical ability, accomplished the thesis objectives, and are already partially published (except Chapters 4 and 6) in relevant journals. They are novel and provide a relevant contribution to crayfish biology, particularly Chapters 2-5. Chapters 3 and 4 show how pollutants can affect the burrowing behaviour and offer interesting starting points for future research on other crayfish species. Chapters 2, 5 and 6 confirm the high invasiveness of marbled crayfish, even on other invasive crayfish. Chapters 5 and 6 show the importance of investigating the relationship between NICS (a still understudied topic) to better understand their behaviour in the wild (and provide useful hints for their management).

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***OVERALL COMMENTARY ON THE PhD THESIS***

**Please write comments in extent of 1-2 pages:**

The thesis is composed by five main articles, three already published (in high impact factor journals). I don't have any particular comments to be addressed for Chapters 2, 3 and 5 that are already published. The introduction (Chapter 1) and the discussion (Chapter 7) are comprehensive; Chapters 4 and 6 (not yet published) present interesting results on effect of herbicides and interspecific competition on burrowing behaviour. I have some suggestions for the Chapters 1,4,6,7 listed below.

Pages 9-10, 13-15, 17-26, 112-122: please justify the text- Should also Chap 4 and 6 have justified text as their abstracts?

Page 9, end of first paragraph: I would add also that crayfish are important as model animal for research at Unis and schools (see for example in North America).

Page 9, beginning of second paragraph: burrow are important also for females carrying eggs.

Page 9, third paragraph: Engaeus in italics; I guess it is an „alternative classification“, not „alternate classification“.

Page 10, 1.2, end of paragraph: pollution is reported twice; I would include overharvesting.



Page 10, 1.2.1, second paragraph: not sure „become taxed“ is the best expression; tax usually is used for mental efforts. Maybe „are so affected that recovery..“

Page 13: „1.3 Crayfish burrowing behaviour“ not „1.2. Crayfish burrowing behaviour“.

Page 13, 1.3.1, end of page: please add a space before „Removal“.

Page 14, second paragraph: the cast without the t of the in capital.

Page 14, 1.3.2: I guess it is „...McClain (2010) placed a single crayfish...“

Page 15, last paragraph: *F. limosus* in italics; moreover, is it „emerging invader marble crayfish“? Moreover, it would be interesting to report here the species used for the thesis and sum up the reason of their selection. Finally, I would better present the last objective in the light of burrowing behaviour.

Table 1: please adjust in order not to have the word „disadvantages“ divided; moreover, it is not species of burrower (no specific species are reported), „burrowing animals or taxonomic groups“ would be better.

Chapter 3: it would be interesting to replicate this experiment with the marbled crayfish, spinycheek and calico crayfish.

Chapter 4: has it been already submitted to any journal?

Chapter 4, methods: please explain the reason to have selected 28 days for exposing crayfish to the herbicides; discussion: while a stress response in males but not in females?

Chapter 4, page 55, last line m<sup>3</sup> with 3 in apex.

Chapter 4, page 58, almost at the end: Guo et al. (2019a).

Chapter 4: where is Table 1? I guess the actual Table 2 is Table 1, and Table 3 is Table 2, so they should be correctly cited in the text.

Chapter 4, caption Fig. 1: it should be „Number of burrows in the different groups and sex“ and the n per group should be reported in the caption. Please report the n also in Fig. 2.

Chapter 6: has it been already submitted to any journal?

Chapter 6, abstract: „...was significantly higher than that of spiny-cheek crayfish *F. limosus* in general“. What does „in general“ mean here? Please better specify. Moreover, please M of marbled crayfish in capital as it is at the beginning of a sentence (the same few lines below).

Chapter 6, page 92: please replace „Studies comparing the interactions of NICS with one another“ with “Studies assessing the interactions between NICS are appearing”. In the same paragraph, few lines below please put *Faxonius virilis* in italics. Also *F. limosus* at the end of the page should be in italics.

Chapter 6, page 93, 6.2.1: *Cyprinus carpio* in italics. Moreover, aquaria (not troughs) were cleaned daily.

Chapter 6, page 95: the name of the species should be in italics. “...the rate of marbled crayfish shelter occupancy dominance was higher than that of spiny-cheek crayfish. The suggests..” The suggests maybe is “This suggests”?

Chapter 6, page 96: please consider Holdich & Black 2007. Aquatic Invasions 2:1-15 for *F. limosus* burrows: the authors reported different type of burrows for the species.

Chapter 6, half page 96-before conclusion: this part of the discussion is a repetition of the first part of the discussion. Please remove it.

Chapter 6, page 97: I would say that “The distribution of marbled crayfish *P. virginialis* in natural water bodies is expanding rapidly” as showed by the continuous new records in Europe.



Chapter 6: also here table numeration starts with 4. Where are Tables 1,2,3? Please correct in the entire manuscript.

Chapter 6, table 7: were strong contacts also considered? They indicate the aggressiveness of the individuals; table 8: medians and interquartiles should be reported when using non-parametric statistics.

Discussion, pages 111-112: please the scientific name of species should be in italics. Moreover, I suggest not using „our research, we...” but „my research, I..” or similar. *Perca fluviatilis*, not *Percha fluviatilis*.

Discussion, page 112: I think that it would be noteworthy to discuss the implications of the findings in Chap. 3 & 4 on invasive alien species management. Moreover, do these pollutants have an effect on agonistic behaviour of crayfish?

Discussion, page 114, last paragraph: please add a space between higher and mortality.

Abstract: please the scientific name of species should be in italics. As for discussion, please do not use „we”. Should the abstract report references?

## ***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**

Florence, 2020, July 5th

Elena Tricarico