



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Viktoriia Malinovska, Ing.	First name(s), surname, titles of supervisor: Pavel Kozák, prof. Ing. Ph.D.
Title of PhD thesis: Monitoring of crayfish reaction to the different stimuli: are the crayfish good bioindicators?	

REVIEWER:

Surname: Tricarico	Institution: University of Florence, Italy
Name: Elena	
Titles: Dr.	E-mail: elena.tricarico@unifi.it
Please describe your professional relationship to the PhD student: I met once at the IAA conference (2022)	Please describe your field of expertise: Biological invasions, behavioural ecology of crayfish, management of crayfish and other aquatic alien

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**):

Considering the current increase of pollution in fresh waters involving different toxic substances, biomonitoring is still a crucial issue, and crayfish represent optimal model organisms to investigate this topic for their ecology, life history traits, possibility of bioaccumulation and fundamental role in aquatic ecosystems. Besides traditional approach of biomonitoring and evaluation of lethal effects, there is a growing demand to assess the sublethal effects (i.e. those altering the behaviour and possibly leading also to the death of individuals), using indeed animal behaviour as a reliable tool and as non-invasive methods to explore them. Despite this, the studies on sublethal effects using a behavioural approach in aquatic animals are still few and the review carried out by the candidate in Chapter 1.2 well pointed out this and the need to more research in this direction. Overall, the thesis addresses some of these gaps (e.g. using a behavioural approach in the research) and also evaluates for the first time in signal crayfish the effects of chlorine dioxide, showing a disturbance in the circadian cardiac rhythm, and of organic UV filters, that induced changes in movement, activity time and heart rate. The results are noteworthy and important, improving the knowledge on the effects of some pollutants on crayfish, particularly the sublethal effects, and confirming the possibility to use crayfish as bioindicator coupling behaviour and physiology on a short and long term. The level and quality of the thesis are very good, as also testified by the three



already published articles in high impact factor journals. The work is very interesting and timely for the scientific pertinent field, and is relevant for pure and applied research, as it provides solid evidences to positively use non-invasive methods for biomonitoring research in crayfish, that can be applied in further studies on other pollutants (also combining pollutants in a same study).

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 three main objectives well elucidated at the end of Chapter 1, for whose achievement the candidate conducted a review and several laboratory experiments. Overall, the thesis is a solid work, well and clearly structured. The methods are appropriate, clearly illustrated and explained. The candidate mostly used a combined approach of behaviour (i.e. locomotion) and physiology (i.e. cardiac activity) to assess the sublethal and lethal effects of the tested pollutants, confirming the efficacy of this choice. The invasive signal crayfish *Pacifastacus leniusculus* has been used as model organism to facilitate the research. The English results fluent and well written. The results are well presented, discussed with a good very critical ability, accomplished the thesis objectives, and are already almost published (except Chapter 1.2) in international journals with Impact Factor. As mentioned above, they are all novel and provide a relevant contribution to the field of biomonitoring.

Chapter 1.2 provides an important and exhaustive review on the studies conducted to evaluate the effects of pharmaceutically active compounds, pesticides, and heavy metals on freshwater crayfish: it is a very important state of the art not only for the thesis but also for future research in the area.

Chapter 2, 3, 4 show the effects of some pesticides, organic UV filters and water treated with chlorine dioxide, highlighting their different effects on short and long term on heart rate and movement. All these chapters, besides the novel results, offer interesting starting points for future research on the topic.

OVERALL COMMENTARY ON THE PhD THESIS

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



The thesis is composed by four main articles, three already published (in high impact factor journals) and one ready for the submission. I don't have any particular comments to be addressed for Chapters 2, 3 and 4 that are already published.

For Chapter 1.2 and Chapter 5 I have a few comments to be addressed (please see below).

Page 8: Styriš et al., 2007: please put 2007 between brackets

Page 8: please consider that currently also the use in research for non-native crayfish is subject to regulation (i.e. permit if they are in the list of species of Union Concern linked to the EU Regulation 1143/2014)

End of page 8: please correct "an unique organism"

Page 9: please refer to Chapter 1.2 at a certain point while presenting the chapters and their content

Chapter 1.2: what about biopesticides?

Chapter 1.2, page 23: please mention also the biomagnification (and threat) for other predators in the food web

Chapter 2-4: for future studies please remember to report the permit to use signal crayfish (as I guess it is required also in Czechia) as it is the Union list

Page 80: could you please hypothesise any management consequences with this increased locomotory activity?

Page 80: please in the sentence "This suggests that the impact of these pesticides extends beyond immediate behavioral responses and may affect the physiological health and overall fitness of crayfish populations" mention the possible effect on the entire community.

In the abstract please mention Chapter 1.2.

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**

Florence, 25/06/2023

.....

Date and place

.....

Name and signature