



**Confidential**

**Review of USB FFPW PhD Thesis**

<b>First name(s), surname, titles of the PhD student:</b> Hamid Niksirat Hashjin, M.Sc.	<b>First name(s), surname, titles of supervisor:</b> Assoc. Prof. Dipl.-Ing. Pavel Kozák, Ph.D.
<b>Title of PhD thesis:</b> Biology of reproduction in the crayfish	

**REVIEWER:**

<b>Surname:</b> López Greco	<b>Institution:</b> University of Buenos Aires Cdad. Univ. C1428EHA Buenos Aires Argentina
<b>Name:</b> Laura S.	<b>E-mail:</b> <a href="mailto:laura@bg.fcen.uba.ar/">laura@bg.fcen.uba.ar/</a> laura_lopez_greco@hotmail.com
<b>Titles:</b> Dr. and Professor	
<b>Please describe your professional relationship to the PhD student:</b> I have not relationship with the student. This thesis is my first contact with the student	<b>Please describe your field of expertise:</b> Reproductive Biology in decapod crustaceans (mainly freshwater crayfish genus <i>Cherax</i> , eubranchyuran crabs and caridean/penaeid shrimps) Actually I am doing some research on growth and nutrition in freshwater decapod crustaceans

**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**):

This PhD thesis is an original analysis about some features of crayfish reproduction. This is well written, clearly presented and well discussed in all chapters. This discussed theoretical mains points and this applied aspects mainly about aquaculture. In this way the results could have important impacts for astaciculture.



### ***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 level of elaboration is properly and fine. The introduction and the chapters are well structured and sequentially well presented. The approaches to solve the questions are adequate in all cases. The results are very well published and the next papers will be also of an excellent quality without any doubts.

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

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

1- First of all I think you have to change the title of you PhD thesis. Your thesis is not about ***Biology of reproduction “in the crayfish”***. The crayfish does not exist. Your thesis is about at least Astacidae and Cambaridae. There is another important group you are talking about (and clearly very different from other families), the Parastacidae. I can accept your “Biology of reproduction” although you are only researching in a few features of reproductive biology. Your title is more for a book/revision than for a PhD thesis.

2-Be more careful with this statement (page 7) “*Female crustaceans store the spermatophores for long periods in their thelycum, and the eggs do not have to be laid immediately after mating (Nagaraju, 2011)*”. This is a very broad statement that does not represent the female crustaceans as a whole. Long *versus* short periods depends much of the species and many species have to lay eggs immediately after mating. Within decapods caridean shrimps, for example do not store spermatophores for much time. They practically lay eggs in the next minutes/hours after mating. I will re-write this phrase.

In the aims (page 14): Why you choose 6 species to study and compare ultrastructure of spermatozoa and then you choose one for morphological changes of the spermatophore wall and spermatozoon during post-mating storage and other to study egg ultrastructure and its morphological changes during egg activation? I believe you should explain it to the reader (the criterion for the election).

Chapter 1: All information is clearly described and developed. The bibliography is adequate and complete. The importance of basic biology in crayfish reproduction is fine pointed out. Although many studies have been conducted in spermatozoa morphology this thesis provided new and compared information. The other topics discussed (chapters 3 and 4) are really pioneer studies.



Chapter 2: Ultrastructure of crayfish spermatozoa. Both papers are excellent and published in journals with good impact factor. I have no comments to do about them. Just congratulations!. The discussion and transmission electron micrographs are really fine.

Chapter 3: Post-mating morphological changes. This future paper will be very nice and complete. I only suggest to complement the electron microcopy point of view to perform some histological/histochemical analysis (PAS, MASSON for example) to characterize the biochemical changes in the spermatophore layers after extrusion. Maybe not for this instance (thesis!) but you can explore/dive on this after your PhD defense.

Enclosed is the reference of a paper about this kind of analysis that maybe gives you some ideas (ZARA, F.J., TOYAMA, M.H., CAETANO, F.H. and LÓPEZ GRECO L.S. 2012. Spermatogenesis, spermatophore and seminal fluid production in the blue crab *Callinectes danae* (Portunidae). *Journal of Crustacean Biology* 32 (2): 249-262)

**Table 1 is a fine integrative and comparative approach of the spermatophore layers and its changes.**

Chapter 4. Egg activation. The results of present chapter showed the morphological changes of first envelope of egg and formation of a second envelope in perivitelline space and a partially egg cortex after egg activation. Together with chapter 3, this is the most impacting approach and knowledge from this PhD thesis.

Chapter 5. General discussion has a correct and integrative approach.

### **FINAL RECOMMENDATION**

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

Buenos Aires, Argentina, June 10, 2014

Dra. Laura Susana López Greco



Fakulta rybnářství  
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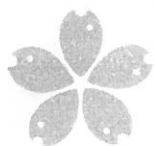
Buenos Aires, Argentina

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Date and place

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Name and signature



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**REVIEWER:**

<b>Surname:</b> Ráb	<b>Institution:</b> Ústav živočišné fyziologie a genetiky AV ČR Rumburská 89 277 21 Liběchov
<b>Name:</b> Petr	<b>E-mail:</b> rab@iapg.cas.cz
<b>Titles:</b> Prof. Ing., DrSc.	
<b>Please describe your professional relationship to the PhD student:</b> None	<b>Please describe your field of expertise:</b> Fish genetics, cytogenetics and taxonomy/biodiversity research

### **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**):

Submitted PhD Thesis deals with the detailed morphological/anatomical study of ultrastructure of spermatozoa of six crayfish species, either native or invasive, occurring in the territory of the Czech Republic and compare results/observations with the current knowledge in this specific field. The level of science is of high quality, the elaboration of studies is very careful and surely the results represent very good international quality as evidenced by already published papers in good journals. Since high significance of development of crayfish reproductive biology associated with general need of crayfish aquaculture worldwide the Thesis represents excellent contribution to this field both as basic and applied research with potential of future development.



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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, logic 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**):

Submitted PhD Thesis is based on 4 studies, 2 already published (and hence independently peer reviewed) and 2 ready-to-submit manuscripts (though it is not clear to what journals they will be directed). All these four studies are focused to the ultrastructure of spermatozoa of six crayfish species and hence Thesis are desirably thematically restricted which evidently enabled to formulate Introduction as condensed, focused and very informative part. Objectives were evidently accomplished, the language is clear, statements and formulations are logic, structuring of the text also logic. At formal level, Thesis reaches nearly perfect state without typos.

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

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

Submitted Thesis represents very clear, focused document based on 2 published and 2 ready-to-published studies, those already published in quite good journals. Thesis contains condensed, but very clear and well logically arranged Introduction. The same applies for Discussion which is desirably short, condensed but on the other very informative commenting observations and results. The data about microstructure of crayfish spermatozoa are important not only for basic research but also applicable in crayfish reproductive biology. Thesis is formally at the high level, so it is difficult to comment on some problem contained in it. The only question deals with possible cryopreservation of crayfish spermatozoa. Are there some experiments?

## **FINAL RECOMMENDATION**

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 **PhD Thesis can be recommended with reservations for defence**  
 **PhD Thesis cannot be recommended for defence**

Liběchov 26. 5. 2014  
Date and place

  
Petr Ráb  
Name and signature