



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Azadeh Mohagheghi Samarin, M.Sc.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Tomáš Polícar, Ph.D.
Title of PhD thesis: Alteration of mRNA abundance, oxidation products and antioxidant enzyme activities associated with fish oocyte ageing	

REVIEWER:

Surname: Bobe	Institution: French National Institute for Agricultural Research INRA · Fish Physiology and Genomics Institute (LPGP) France
Name: Julien	
Titles: Prof.	E-mail: julien.bobe@inra.fr
Please describe your professional relationship to the PhD student: host for a short visit (2 months)	Please describe your field of expertise: fish reproduction and genomics, fish egg quality in aquaculture

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 PhD conducted by Ms Azadeh Mohagheghi Samarin deals with an important matter, the impact of post-ovulatory ageing on egg quality in aquaculture species. Overall, the PhD project relies on a significant amount of work based on the molecular and enzymatic characterization of post-ovulatory egg ageing in several species. Even though I do not agree with all the conclusions, the PhD thesis has gathered an important amount of solid and original data based on the use of methodologically well conducted and well designed experiments. The PhD thesis does provide original and useful information in several fish species, including species used in aquaculture or as model species in basic biology research. The PhD work has already generated several publications (including a review) in international peer-reviewed journals and two manuscripts are currently being submitted (or about to be). Together, the work of Ms. Azadeh Mohagheghi Samarin will therefore significantly contribute significantly to our knowledge of post-ovulatory oocyte ageing in fish not only in terms of egg quality dynamics but also in terms of underlying mechanisms. While the analysis of maternal messenger RNAs over time did not necessarily provide that much new information on the evolution of egg transcriptome during ageing, the analysis of enzymatic activity is in contrast much more informative. The main conclusion of the work "i.e. Oxidative stress is not the main initiator or promotor in the progress of fish oocyte ageing (page 95)" is indeed a major



contribution to the field. Similarly, the data on the evolution of GPX activity over time during ageing is also very original and an interesting contribution to our understanding of the impact of post-ovulatory ageing on egg quality, even though the observed in GPX activity is more likely a consequence, rather than a direct cause, of the ageing process but could still very well playing a major role in the overall drop of egg quality.

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 PhD thesis is extremely well written and the structure is based on the use of scientific articles as main chapters of the manuscript. This structure, based on existing and independent bodies of work, makes the reading of the document very easy, as each chapter is an independent story in a different species. A last chapter, in which a comparative analysis among the different species used would have been enjoyable and useful and was somehow expected given the diversity of the biological models investigated. Nevertheless, the overall objectives of the work are achieved and the experiments are designed in a way that the PhD will yield an important number of scientific publications.

The methodology is appropriate and the characterization of egg quality is done very carefully and thoroughly (including survival at different key steps of development as well as the recording of larval malformations), which is especially important in studies dealing with egg quality. Of note, the use of different species that has generated a need for the appropriation of the biology of the different models used. The analytical methodologies used are rather classical and rely mostly on quantitative RT-PCR (QPCR) as well as biochemical/enzymatical kits or protocols. The QPCR is methodologically well conducted although the normalization procedure is not consistent among the different species. A large-scale (i.e. genome-wide) analysis would have been of course more powerful but it is noteworthy that this type of analysis cannot be performed routinely and requires appropriate funding and bioinformatics support. The monitoring of enzymatic activity is more original and has yielded useful information both in terms of the evolution of GPX activity in tench, but also in challenging the dogma that oxydative stress might be an important determinant of egg ageing.



OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

As indicated above, the PhD thesis succeeds in many ways in achieving the objectives of the project and more specifically:

- In providing “reference” work on the timing of post-ovulatory ageing in different fish species including that are important in aquaculture, including a thorough analysis of embryonic/larval survival and malformations
- In generating data on the evolution of maternally-inherited messenger RNA abundance throughout the post-ovulatory ageing phase in the above mentioned species, as well as tentative correlations between the overall developmental competence of the egg and mRNA abundance of specific genes. The identity of the genes is likely to provide novel information on the nature of the molecular mechanisms impacted by the ageing process. Further analysis of these genes during development in eggs at varying ageing times will be very useful and will greatly benefit to the scientific community working on fish egg quality.
- In providing original data on the enzymatic activity of egg during ageing, and more specifically in challenging the dogma that oxidative stress might be a strong determinant of the drop of egg quality during ageing. Of courses, complementary analyses will be required to fully evaluate the contribution of oxidative stress to the drop of egg quality during post-ovulatory ageing.

My main concern would be the interpretation of several results or the use of specific terms and more specifically:

- The term “higher” vertebrates is not appropriate, even though present in many scientific journals. From a phylogenetic standpoint, mammals are not higher than fish.... They are exactly at the same level of evolution. This should be avoided as much as possible.
- It is also inappropriate, in my opinion, to write “up-regulation of expression” when referring to the evolution of mRNA levels/abundance during ageing. Indeed, it is likely that no transcription occurs in metaphase 2 oocytes. Therefore, the so-called increase is rather linked to the relative of abundance of other mRNAs, and subsequently not a true increase.
- In my opinion, it is also difficult to speculate on the evolution of mRNA abundance during ageing as there is no clear link between mRNA abundance and protein synthesis in metaphase 2 oocytes. Indeed, a specific mRNA could very well be degraded after being translated into a protein.



Despite these minor comments, this PhD work was overall nicely done and fully deserves oral defence without any limitation. I therefore fully recommend this thesis for defence.

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

Rennes, June 25th, 2018

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Date and place

Julien Bobe

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Name and signature



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Title of PhD thesis: Alteration of mRNA abundance, oxidation products and antioxidant enzyme activities associated with fish oocyte ageing	
REVIEWER:	
Surname: Kucharczyk	Institution: University of Warmia and Mazury Olsztyn, Poland
Name: Dariusz	
Titles: Prof.	E-mail: darekk56@gmail.com
Please describe your professional relationship to the PhD student: I do not know the PhD student personally. I did not conduct any research with PhD student. I only know some scientific manuscripts by PhD student, because they are related to my professional interests.	Please describe your field of expertise: My professional interests focus on various issues related to artificial reproduction of fish. My topics are: artificial reproduction of wild fish, especially cyprinids, artificial reproduction of percid fishes, quality of gametes, in particular oocytes in connection with aging of oocytes.

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 doctoral dissertation done by Azadeh Mohagheghi Samarin entitled "Alteration of mRNA abundance, oxidation products and antioxidant enzyme activities associated with fish oocyte ageing" is a part of the modern research. The problem associated with the aging of fish oocyte is now one of the scientific tips of modern aquaculture. It is also a problem that is very important for aquaculture production. The processes occurring during oocyte aging negatively affect not only the survival of embryos but also the quality of offspring, causing many changes in both the genetic material and the deformation of the body. All studies that allow



you to study and understand this process are extremely important and useful from both a scientific and a practical point of view. In this approach, the research undertaken by PhD student, fit exactly in this very important scope of modern research.

Research on oocyte aging issues has been conducted intensively and comprehensively for the last few years. Initially, these were issues related to chromosome aberrations, the survival of embryos (biological quality of oocytes) or the appearance of developmental deformities. However, understanding of all the processes occurring in aging oocytes requires further research. And exactly this trend is included in PhD student's research on alteration of mRNA abundance and oxidative stress.

Research conducted by PhD student should be considered original, very modern, very important from the point of view of science and aquaculture development. In my opinion, they will have a huge impact on both science development and aquaculture production.

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 PhD thesis entitled "Alteration of mRNA abundance, oxidation products and antioxidant enzyme activities associated with fish oocyte ageing" by Azadeh Mohagheghi Samarin is constructed correctly as possible in terms of the requirements for such works. The dissertation is divided into six chapters. The aim and specific objectives are presented clearly and intelligibly. All the proposed objectives of the dissertation were carried out. Chapters are arranged in a logical and appropriate way. The work ends with a discussion of the results obtained during studies and conclusions.



The doctoral dissertation includes an Introduction with aims, which chapter is strengthened with a review paper (accepted in Reviews in Aquaculture). Next, the author presents in the next chapters the results of studies obtained in African catfish (published paper), common ten (published paper), common carp (manuscript), goldfish (manuscript) and Discussion with conclusions.

The quality of published papers and manuscripts presented in this dissertation are very high.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The doctoral dissertation entitled "Alteration of mRNA abundance, oxidation products and antioxidant enzyme activities associated with fish oocyte ageing" by Azadeh Mohagheghi Samarin is well done, with correct structure and high scientific quality. The research was done on few freshwater fish species, which has commercial value and might be cultured indoor (African catfish) or in pond culture (common tench, common carp, goldfish). One of the problem in intensive aquaculture is quality of gametes. In the case of spermatozoa quality and management, many research were done and described. Much less was done and published in the case of oocytes. In warm-water species, such as used in present study, the quality of oocytes and their ageing process is very important for commercial aquaculture, due to very fast ageing in comparison e.g. with salmonids. Low quality of eggs, including ageing process, caused by decreasing of embryo and larvae survival and quality, body deformations, chromosome aberrations and many others. So, the understanding the process of oocyte ageing, might be very useful for example in procedures of short-term oocyte storage before fertilization without decreasing of their quality.

The subject of present PhD dissertation entitled "Alteration of mRNA abundance, oxidation products and antioxidant enzyme activities associated with fish oocyte ageing" by Azadeh Mohagheghi Samarin is very important both



scientifically and for field aquaculture. The quality of published papers (3 papers in "Reviews in Aquaculture" and "Aquaculture Research") and manuscripts (2) is very high. As the same, the General Introduction, Discussion and Conclusions are in the same quality.

Once again I would like to emphasize a very high scientific value assessed doctoral dissertation. In addition, many respondents also have a practical aspect and can be successfully used in aquacultural practice.

I also apply to the Scientific Council of the Faculty of Fisheries and Protection of Waters of the University of South Bohemia in Ceske Budejovice, for the **award** assessed dissertation entitled "Alteration of mRNA abundance, oxidation products and antioxidant enzyme activities associated with fish oocyte ageing" by Azadeh Mohagheghi Samarin.

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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

Osaka, 4.5.2018
Date and place

Dominik Kudravný
D. Kudravný
Name and signature