



Fakulta rybnářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice
Czech Republic

Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Roman Franěk, Ph.D.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Martin Pšenička, Ph.D.
Title of PhD thesis: Germ stem cell manipulations as a tool to manage and produce isogenic lines in fish	
REVIEWER:	
Surname: Dabrowski	Institution: The Ohio State University USA
Name: Konrad	
Titles: Dr.	E-mail: dabrowski.1@osu.edu
Please describe your professional relationship to the PhD student: Dissertation reviewer	Please describe your field of expertise: Fisheries and 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**):

This work, particularly because the use of the model species, zebrafish, has enormous implications for both the basic science of vertebrate genetics and aquaculture as well as the applied science of genetic engineering. The originality of the work is unquestionably demonstrated by the acceptance of the papers that are part of the dissertation in various prestigious scientific journals, such as PLOS and Theriogenology.

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**):

Specific objectives are listed on page 19 and explicitly covered in the following chapters (there is a typo error in objective 3). Although one of the overarching goals of the dissertation is to apply the methods developed in this work to produce isogenic lines of fish, the latter aspect is rather a follow



up task on its own and not necessarily to be covered in the separate chapter.

In Chapter 4 the author claims sterility in morphants of interspecific transplantation based on evidence in the literature (Goto et al.) (page 88) but I missed the data related to this specific experiment. Particularly in the light of carp x goldfish hybrids being fertile (female progeny only) and producing diploid eggs when backcrossed to paternal species (Gomelsky et al. 2012). In favor of his argument the author cited (page 155) the work of Gomelsky et al. (2016) (no journal reference was given) to provide evidence of aneuploidy in the polyploid carp ovary. Earlier work of Gomelsky et al. (1988. Dokl.Akadem.Nauk SSSR, 310) was not mentioned where he documented fertility of hybrids and triploids.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The review (Chapter 2) is the only “weaker” part of the dissertation. The germ technologies section description does not include key references (salmon, trout work) but covers some “dubious” results from Lacerda et al. It is unclear if Fig. 1 is a misconception of how the cell structure of the blastula stage appears or an unfinished graphical depiction of that. It appears the cells are only located in the germ ring. The work of the Liu Shaojun lab from Hunan Normal University, Changsha (over 100 publications), who established 3 autotetraploid and 7 diploid multigenerational lineages (14-18 generations) through distant hybridization was not covered at all (Wang et al. 2019. Science China, Life Sciences 62: 22-45; the most recent review).

Some statements are too bold and the author ought to know better. For instance, he declared (page 6) that “somatic cells are not capable to pass their genetic information to the progeny”. John Gurdon was awarded the Nobel Prize in Physiology and Medicine in 2012 for a discovery made 50 years earlier that nuclei from mature frog intestine cells transplanted to enucleated eggs can be reprogrammed to pluripotent cells and then begin embryonic development and produce tadpoles (Gurdon 2013. Development 140).

My question for the author would be, what value does using triploids for these transplantations have over zebrafish x pearl danio hybrids, as proposed and validated by Wong et al. (2011)? The viability of triploids is still compromised in comparison to diploids and requires additional work. Perhaps they are concerned that hybrids would not readily mate naturally with pure zebrafish? It was recently confirmed that hybrids will mate with zebrafish. It would be an enlightening experiment to transplant ovarian cell suspension into hybrids.

One of the concerns, which is general in nature, is criteria defining multi-authorship in publication and contribution of the individual author. For example, in chapter 2 the senior author declared that his contribution was “about 70%”, so that leaves another six authors with an average contribution of 5%. Where is the limit of how intellectual contribution should be evaluated (review paper)? If the second author contributed 9% perhaps one of the authors was responsible for 1% of the effort. The idea and emphasis of multi-authorship should not be abandoned but must be defined and be meaningful and understood by both authors and readers. This is particularly important for PhD candidates, and the next generation of scientists must become critical of this, relatively recent phenomenon, of propagation of authorship in scientific publications that are not based on merit.



Overall I evaluate Mr. Franek's dissertation as outstanding work and grade this in the top 5% of the PhD dissertations I have reviewed in the past 30 years (over 30 PhD's). I recommend that the committee should allow the candidate to proceed to the next step of the process.

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

July 23, 2019 Columbus, Ohio USA

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Date and place

K. Dabrowski

.....

Name and signature



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Roman Franěk, Ph.D.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Martin Pšenička, Ph.D.
Title of PhD thesis: Germ stem cell manipulations as a tool to manage and produce isogenic lines in fish	
REVIEWER:	
Surname: Rodriguez	Institution: Spanish Institute of Oceanography (IEO)
Name: Vanesa Robles	Spain
Titles: Dr.	E-mail: robles.vanesa@gmail.com
Please describe your professional relationship to the PhD student: No relationship	Please describe your field of expertise: Reproductive Biology Cell and Molecular Biology and Cryobiology

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 focused in two fresh water species: carp and zebrafish and it covers several aspects of germ cell biology, including: cell manipulation, cryopreservation and germ cell transplantation.

Research on teleost germ cells is not only relevant for broaden our basic knowledge in Reproductive Biology but it also allows the development of biotechnological tools that could be applied in Aquaculture Industry providing clear production benefits. Therefore I consider that results derived from this PhD thesis could have an important impact in the fields of Aquaculture and Reproductive Biology from both, basic and applied perspectives.

The international dissemination of the scientific results is well proved, considering the numerous presentations in international meetings that have been derived from this thesis. The hosting group is composed by internationally renowned scientists very active in publication of relevant papers. In fact, as a result from this thesis relevant articles have been published in multidisciplinary journals, such as PLoS ONE or in journals relevant in specific areas such as Cryobiology.



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**):

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

This thesis is composed by a high scientific quality original manuscripts and articles. It is well structured and comprehensible for the reader.

From my point of view, the selection of species employed in this thesis is very appropriate. In one hand, zebrafish is a good model species, which is crucial for developing new techniques that could be subsequently transferred to relevant aquaculture species. On the other hand, common carp is an interesting species from aquaculture point of view. Moreover, as it has been stated in the introduction, results could be also transfer to a broad range of fish.

The thesis is organized in 8 chapters:

The introduction (chapter 1) is well organized, adequate in size and contents. It provides a general overview of the state of the art including relevant bibliography. The subtitles and figures facilitated general reading and understanding.

The 6 objectives of this thesis are relevant in the field and clearly presented. The approaches used for their consecution are optimal resulting in the publication in relevant scientific journals.

Chapters 2 to 7 are related to either manuscripts in progress to be published (chapter 5 and 7), manuscripts in revision (chapter 2 and 6) or articles published in peer reviewed relevant journals (chapters 3 and 4). Chapter 2 is an exhaustive and profound revision about isogenic lines in fish that satisfies the objective 1 of the thesis. Chapter 3 is a very interesting study about cryopreservation and transplantation of carp spermatogonia published in a very good multidisciplinary journal. It is interesting to mention that this work is technically very demanding and it requires of high level of expertise. Chapter 4 deals with objective 3, preserving female genetic



resources of carp. Results were published in Cryobiology, which is a renowned journal in the field. Chapter 5 is not yet published however the objective and the results are relevant and their publication is highly probable. Chapter 6 is under review in Theriogenology, a relevant journal in reproductive biology, and it compares the efficiency of cold shock and heat shock treatments for zebrafish triploid production, demonstrating, after transplantation experiments, that triploids are suitable surrogate host. Chapter 7 successfully developed an approach for donor derived gametes production in zebrafish after intraperitoneally grafted blastomeres into swim-up embryos.

Finally, chapter 8 includes the general discussion of the thesis and the PhD student CV:

The general discussion is brief but it is well written and it covers all essential aspects, including the future prospects of germ cell manipulation in fish. PhD student CV clearly showed an excellent dissemination of thesis results in international meetings, with 15 contributions, some of them directly derived from the thesis and some of them as a result of the collaboration with other members of the laboratory. This could be considered a good indicator of the implication of the PhD student in the scientific activities of the group. Regarding to publications on peer-reviewed journals with IF, he has 4 published papers (in 2 of them he is the first author and are directly derived from his thesis) and 2 submitted manuscripts also derived from the thesis. There are 2 more manuscripts (chapters 5 and 7) that are not yet published, which quality makes highly probable their prompt publication. Considering all these meetings and publications it can be stated that there is a very good scientific production derived from the thesis. Moreover, the PhD student performed 2 international stays (INRA, France and Hokkaido University, Japan) which is a valuable aspect for completing his scientific training.

Considering all the above mentioned reasons, I consider that this thesis is a relevant and highly valuable scientific work and I fully support the PhD thesis 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

June 19th, Santander, Spain
Date and place


Vanesa Robles Rodríguez
Investigadora Científica
Organismos Públicos de Investigación
Vanesa Robles
Name and signature