



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Xie Xuan, M.Sc.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Martin Pšenička, Ph.D.
Title of PhD thesis: In vitro culture of sturgeon germ stem cell	
REVIEWER:	
Surname: Troedsson-Wargelius	Institution: Institute of Marine Research, Bergen, Norway
Name: Anna	
Titles: Dr.	E-mail: anna.wargelius@hi.no
Please describe your professional relationship to the PhD student: I do not have one.	Please describe your field of expertise: Reproductive biology of fish.

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 thesis represent a significant addition to knowledge regarding Sturgeon reproductive biology. Here the thesis presents ideas how to solve conservation related issues with declining Sturgeon populations. Where the use of surrogate production could present a major solution to a prompt repopulation of endangered sturgeon species. In the study, the candidate has explored and elegantly established several methodological steps, which can pave the way for an applied protocol for rescue of endangered species. This includes; identification and isolation of type A spermatogonia in sturgeon, in vitro cultivation of germ cells and subsequent implantation into recipients.



Elaboration of the PhD thesis, objectives of the work and deliverables

This is my general view: This thesis in general represents and good quality thesis with regards to the questions, experiments, results and conclusion. There are only scattered language problems in intro and discussion which could be addressed.

Structure: Nice and clear

Comprehensibility: Yes

Logicity: Yes

Originality of the selected approaches: In general, the thesis explores novel methods for sturgeon which in other especially model species are already applied. However, this establishment is as we can see largely species specific and therefore this exploration presented here is crucial to be able to proceed with a full conservation method for sturgeons in general.

Publications: The thesis comprehends four scientific articles of which two are already published in scientific journals. In the other two papers some remaining work, especially regarding writing remains. However, in total this thesis represents an excellent publication record.

Do results correspond to objectives: Yes

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Evaluation of MSc Xie Xuan doctoral thesis

Thesis title: "In vitro culture of sturgeon germ stem cell"

Main supervisor:., Assoc. Prof. Dipl.-Ing. Martin Pšenička, Ph.D.

1. Thesis description

This PhD thesis comprises a general introduction that provides an overview of the topics covered in the thesis, followed four sections which presents the four research papers. The different sections are followed by a discussion and conclusions. In brief, this work presents a method how to cultivate, identify, sort spermatogonia A in sturgeon and briefly presents transplantation into surrogate recipients. The approach and methodology are appropriate, and the results are generally sound and satisfactorily discussed.

2. Thesis evaluation

The background section is up to date with numerous relevant and recent publications refereed to. It gives a good introduction to the subject. The thesis provides an up to date overview of the most important subjects dealt with in the subsequent papers. Unfortunately, the text suffers from numerous grammatical errors which should be corrected I recommend help form a native English speaker to get rid of these errors. The Introduction is followed by the four studies performed in the thesis and ends with a discussion.

Paper I: *Spermatogonial Stem Cells in Fish: Characterization, Isolation, Enrichment, and Recent Advances of In Vitro Culture Systems.* Xuan Xie, Rafael Nóbrega, Martin Pšenička *Biomolecules.*



2020 Apr; 10(4): 644. This is a review paper published this year. The review nicely summarizes the current knowledge on spermatogonial stem cells in teleosts. This work represents a good starting point for anyone who would like to get a current overview of this field of fish biology.

Paper 2. *Optimization of In Vitro Culture Conditions of Sturgeon Germ Cells for Purpose of Surrogate Production* Xuan Xie, Ping Li, Martin Pšenička, Huan Ye, Christoph Steinbach, Chuangju Li, Qiwei Wei *Animals (Basel)* 2019 Mar; 9(3): 106. This is clearly the main paper of thesis. Here the candidate has established a method for sturgeon (sterlet) germ cell culture. Optimal media conditions have been tested and cells have been kept in cultivation for at least 40 days. In addition cultured germ cells from sterlet were then implanted in to Russian sturgeon, where cells of these were detected after 4 months.

Paper 3. *Fluorescence-activated cell sorting of sterlet germ cells based on light scatter properties* Xuan Xie, Galina Kislik, Roman Franěk, Michaela Fučíková, Christoph Steinbach, Tomáš Tichopad, Martin Pšenička *Manuscript*

In this paper FACS was established as a method to sort out spermatogonia A, from freshly prepared testicular cell suspension from sterlet. The methods could significantly sort out spermatogonia A and presents a promising method to use prior to establishment of either cell culture or transplantation.

Paper 4. *A novel monoclonal antibody for identification of type A spermatogonia in sturgeon* Xuan Xie¹, Kensuke Ichida², Yoshiko Iwasaki-Takahashi², Goro Yoshizaki², Christoph Steinbach¹, Michaela Fučíková¹, Roman Franěk, Martin Pšenička¹. *Manuscript*

In this study it was identified an antibody which specifically recognize sterlet spermatogonia A. This antibody could be used to efficiently sort out spermatogonia as is done in paper 3. However, what would be very interesting and that is not presented in the paper is to identify this protein, I miss this discussion.

3. Conclusions

The thesis covers all relevant subjects for the thesis, the text is easy to follow, figures are easy to interpret. The thesis could have gained from English improvement, and maybe addition of a few discussion themes. However, the thesis comprises four research papers that contribute significantly the knowledge and methods associated with germ cell culture methods in sturgeon. Two of the four papers have been published peer reviewed scientific journals, which illustrates the high academic standard of this work. While the two manuscripts could be combined into one paper, as the content is related. It is my conclusion that MSc Xie Xuan dissertation meets a high academic standard and is suitable for defence to obtain the PhD degree.

FINAL RECOMMENDATION



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

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

03.07.2020 Bergen

Date and place

Anna Troedsson-Wargelius

Name and signature