



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Hilal Güralp, M.Sc.	First name(s), surname, titles of supervisor: M.Sc. Taiju Saito, Ph.D.
Title of PhD thesis: Embryo development and transplantation of primordial germ cells in pikeperch <i>Sander lucioperca</i>	
REVIEWER:	
Surname: Lareyre	Institution: Institut National de la Recherche Agronomique Laboratoire de Physiologie et Génomique des Poissons Campus de Beaulieu, 35042 Rennes cedex (France)
Name: Jean-Jacques	
Titles: Dr.	E-mail: Jean-Jacques.Lareyre@rennes.inra.fr
Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: Male reproductive physiology, Spermatogenesis, germ stem cell biology,

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):

Originality:

The studies described in the manuscript entitled “Embryo development and transplantation of primordial germ cells in pikeperch *Sander lucioperca*” provide new basic knowledge on the early development in pikeperch *Sander lucioperca* and its control by environmental factors such as temperature. Temperature change (10°C versus 15°C) affects the success rate of the fertilization, decrease the survival rate, and slow down embryo development. The yolk Syncytial Layer (YSL) and Mid Blastula Transition (MBT) stages are key events for the successful and harmonious development of fish embryos. The study demonstrates for the first time that YSL was initiated between the 510 and 1k stages and that MBT was achieved at the 1k stage in pikeperch *Sander lucioperca*. The studies describe an original behavior of the primordial germ cells during their migration towards the genital ridges.

The studies carried out during the course of the PhD thesis provide also new species specific and practical information for blastomeres manipulation in the pikeperch *Sander lucioperca*. The study determined that the 1k stage is the appropriate competent stage to perform the blastomere transplantation technique (BT). In addition, the study demonstrated that the blastomere transplantation technique can be successfully carried out from donor blastula stage to generate germinal chimera that support donor PGCs survival.



The PhD manuscript provides a well-documented state of knowledge on the biology of primordial germ cells in fish and on the biotechnologies based on their manipulation. Unravelling the mechanisms underlying PGCs determination from blastomeres and migration behaviors and pathways are important issues for human health and agricultural researches.

Researches on germ stem cells biology is a highly competitive and innovative research domain. The present studies used state of the art technique and original tools that are mastered by only few research teams worldwide. Combined with the well recognized zootechnical background, there is no doubt that the research carried out during the course of the PhD program participated to maintain the research team among the top 10 laboratories capable to develop biotechnologies based on the manipulation of germ stem cells in cultured fish species.

Perspectives and impacts for basic and/or applied research:

The present study offers new perspectives for basic researches. The mechanisms underlying PGCs determination from equipotent blastomeres remain to be further investigated because this could give clues to select the appropriate blastomeres capable to generate germinal chimera by BT. In addition, the mechanisms underlying the migration of the PGCs and the amplification of the initial germ stem cell stock in pikeperch *Sander lucioperca* could be further investigated to improve the colonization efficiency of the recipient gonads.

The study demonstrated that BT could be successfully carried out in pikeperch *Sander lucioperca* to generate embryos with donor germ stem cells colonizing recipient gonads. This suggests that BT can be used to generate germinal chimera that produce functional gametes from donor PGCs. In consequence, the work carried out during the course of the PhD program provides a proof of concept that pikeperch *Sander lucioperca* is a suitable recipient for the development of surrogate production and regeneration of valuable genetic resources in perciforms using the BT technique. However, additional challenges will have to be solved to generate sterile recipients and improve the success rate of the BT technique.

stomer

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**):

The thesis manuscript includes a well-documented introduction, a clear description of the objectives, three chapters corresponding to primary research articles describing the experimental design and significance of the results, and a general discussion and conclusion.

The manuscript is well elaborated, clear and easily understandable. However, a minor point is that



English spelling and grammar need to be checked particularly in the introduction and discussion sections.

The illustrations used in the general introduction are well suited and help to the understanding of the text and statements. The different experiments are well designed to address the scientific objectives and technical challenges of the PhD thesis. All experiments followed logically and are appropriate to implement the BT in pikeperch *Sander lucioperca*.

All results, described in three research articles, have been published in a peer reviewed journal (Czech journal of Aniaml Science) with an acceptable impact factor of 0.98.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The manuscript entitled “Embryo development and transplantation of primordial germ cells in pikeperch *Sander lucioperca*” is well structured and includes a general introduction, a description of the objectives, three chapters describing the experimental design and data, a general discussion and a short conclusion.

The general introduction presents first the interest of the pikeperch *Sander lucioperca* for the aquaculture industry. It also provides a synthetic and up-to-date review of the state of knowledge on the biology of PGCs and on the biotechnologies based on the transplantation of germ stem cells in fish. A reference could be added to mention the work of a research team that is leading the research domain (Nobrega et al.2010. PLoS One, 20;5(9). pii: e12808).

The following three chapters described experimental data.

Chapter 2 described new basic knowlledge on the timing of early development in pikeperch *Sander lucioperca* and its control by environmental factors such as temperature. Increasing the incubation temperaure from 15 to 20°C, accelerate the timing of embryo development. In contrast, decreasing the temperature down to 10°C delayed the timing of the embryo development and this could offer more time to carry out BT. However, the survival rate is greatly decreased at 10°C versus 15°C.

Chapter 3 identified the timing and stages of YSL (at 11 hpf between 510-cell and 1k-cell stages) and MBT (15 hpf at 1k-cell stage). It also described the survival rate of BT carried out at different time of early development from 512-cell to sphere stages. The competent stage for BT appeared to be the MBT stage.

Chapter 4 described the migration path and behavior of the PGCs during late embryogenesis of pikeperch *Sander lucioperca* and demonstrated that BT can be carried out successfully to generate germinal chimera.

Experiments described in chapers 2 to 4 were logically connected, well conducted and included technological achievements.

The last section of the manuscript discussed the main data presented in chapters 2 to 4 with a critical analysis of the limitations of the methodologies and interesting comparative analyses.



The strong point of the PhD studies is the originality of the fish model used and the combination of methodologies based on the use of molecular tools encoding for a fluorescent protein to track germ stem cells in living embryos and on the manipulation of blastomeres to generate germinal chimera. The weakness of the present study is that there is no demonstration that the germ stem cells derived from BT are capable to generate efficiently functional gametes.

The data presented in the thesis manuscript offer new possibilities of continuing or extending the research. Possible extensions of these researches could be to improve the success rate of BT (use of fluorescent proteins targeted into the germplasm to specifically label and transfer the blastomeres that differentiate into PGCs), or to develop the transplantation of cryopreserved germ stem cells in perkerperch embryos, fry or adults. Further investigations could be carried out to generate sterile recipient fish.

There is no doubt that the present study opens new lines of research on surrogate production in perciforms.

I would like to congratulate Hilal Güralp for the quality of the thesis manuscript and the clarity of the objectives and statements.

Based on the quality of the experimental data, the pertinence of the discussion and reflection on the significance of the different findings, I do think that Hilal Güralp deserves the title of Doctor in Biological Sciences.

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 15th 2017 at Rennes (France)

.....

Date and place

Jean-Jacques LAREYRE,

.....

Name and signature



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Hilal Güralp, M.Sc.	First name(s), surname, titles of supervisor: M.Sc. Taiju Saito, Ph.D.
Title of PhD thesis: Embryo development and transplantation of primordial germ cells in pikeperch <i>Sander lucioperca</i>	
REVIEWER:	
Surname: Goto	Institution: South Ehime Fisheries Research Center, Ehime University
Name: Rie	
Titles: Dr.	E-mail: Goto.rie.me@ehime-u.ac.jp
Please describe your professional relationship to the PhD student: No relationship	Please describe your field of expertise: Fish reproduction, developmental biotechnology, sex differentiation in 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**):

Pikeperch is an important species for fisheries in Europe. With the total amount of catch decreasing, the need for aquaculture production of pikeperch is becoming apparent. In this PhD thesis, the process of early embryonic development, specification and localization of primordial germ cells and a method for blastomere transplantation were reported for technical development of surrogate propagation as an original research. Pikeperch eggs have a large oil globule in a single mass yolk. The embryonic manipulation of this type of egg is quite difficult and challenging. The information and technique provided here showed the feasibility of embryonic manipulation in percid fish and the possibility of surrogate propagation as an aquaculture technique in future.



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

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**):

In this PhD thesis, research objectives indicated a cogent understanding of the importance of pikeperch aquaculture, the status of current production and the need for sustainable production in this species. Ms Hilal Güralp has published three papers in international journals as a first author. The first two journal publications featured salient information about the embryogenesis and the migration pattern of primordial germ cells using cytological and embryological approaches as new information in pikeperch. The third journal publication provided detailed information of timing for producing germline chimera using embryo manipulation with a molecular method in pikeperch. Each thesis chapter, except the chapters of general introduction and discussion, was based on those published papers, and each was well-organized, independent and provided high quality experimental data with succinct methods and techniques. The procedures were described step by step to accomplish the overall objectives using evidence-based data. Overall, the PhD thesis is well written, brings understanding to the readers and provides clear objectives that are achieved, therefore I recommend this PhD thesis for defence.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The PhD thesis consists of 5 chapters including a general introduction and discussion. The other three chapters were based on the three published journal papers, respectively, therefore most comments below are about chapters 1 and 5.

Major comments:

In chapters 1 and 5, the author was inconsistent using both American and British English. Consequently, there are some misspellings. For example, there are these inconsistencies - localisation, fertilisation, visualise and the like in chapters 1 and 5, but American English was used in the other chapters. I recommend submitting these two chapters to an academic English proof-reader, if the author is not a native English speaker.

In chapter 1.1, the author mentioned the current fish production and the need to increase the production of fish as a protein for sustainable supply in future. Then, the author described the importance of developing aquaculture techniques including surrogate propagation. Some sentences need to be rephrased to improve the flow and to ensure the information is easily understood. Furthermore, some sentences seem to lack impact. Concerning the surrogate propagation in fish, there are a few methods to generate germline chimera as the author mentioned later in the chapter. However, I think the author needs to explain the potency of



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

surrogate propagation and why the focus was on blastomere transplantation for pikeperch aquaculture here.

There are figures in chapter 1 where the author referred to publications in each image. However, it is difficult to understand how or why those references apply. If the image was a part of the publication, it is better to write as "adapted from Güralp et al., 2015" or "based on Güralp et al., 2015". If the schematic illustration was drawn based on the published paper, it could show the references as "summarized from Güralp et al., 2015 and Saito et al., 2013".

Minor comments: Please check the PDF file where added comments are written in longhand.

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 15, 2017

Date and place

Rie Goto

Name and signature