Fakulta rybářství 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: Viktoriia Iegorova, M.Sc.	**First name(s), surname, titles of supervisor:** M.Sc. Taiju Saito, Ph.D.
Title of PhD thesis: Polyspermy produces viable mosaics in sturgeon	

REVIEWER:

Surname: Goto	**Institution:** Nishiura Station, 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:	**Please describe your field of expertise:** Finfish aquaculture, Fish reproductive biology, Fish developmental biotechnology

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)**:

Sturgeons are quite an important species not only for aquaculture but also for their evolutionary aspect as a living fossil. It is well-known that sturgeons possess several micropyles, unlike other teleosts. There were several reports that an atypical division of sturgeon embryos was found, and it was thought to be a result of polyspermy. In this PhD thesis, cytological and embryological methods were applied to investigate this atypical division of sturgeon embryos to discover the mechanism of its occurrence and to discuss the way of fertilization in sturgeons. During the course of this research, it was found that normal development and survival of embryos with atypical division indicate that sturgeons utilize "physiological polyspermy". Furthermore, the mosaic fish, which developed from an embryo with atypical division, retained haploid cells with paternal nuclei. This phenomenon was also shown in the progeny produced from three interspecific parents as a first finding.

This PhD thesis provided novel and valuable findings for basic biology in animals. Furthermore, the new knowledge obtained here will be useful for chromosomal manipulation and propagation for both aquacultural and conservational purposes in sturgeons.

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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, logicality 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, the research objectives indicate a cogent understanding of the importance of sturgeon aquaculture, the status of wild populations, and the need of proper genetic management for both aquacultural and conservational purposes. Ms. Viktoriia Iegorova has published two papers in international journals as a first author. The first journal publication featured salient information about "karyogamy with an additional plasmogamy" of the physiological polyspermy as novel information on sturgeons. The second journal publication provided the first evidence of viable progeny produced by three interspecific parents in sturgeons. Each thesis chapter, except for the general introduction and discussion chapters, was based on these published papers, and each was well-organized and independent while providing 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.

The research objectives are clear and simple; however, I felt that it is better to state the intended purpose and the research goals clearly in the first chapter. There are sentences that are difficult to understand, and some words are misused. Therefore, I strongly recommend that you submit this thesis for English proofreading, especially the first chapter.

Overall, this PhD thesis is well-written scientifically, brings fresh understanding to readers, and provides clear objectives that are achieved; therefore, I recommend this PhD thesis for defense.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Please see a PDF file with some comments.

FINAL RECOMMENDATION

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- [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**

October 12, 2018
Date and place

Rie Goto Rie Goto
Name and signature

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Title of PhD thesis: Polyspermy produces viable mosaics in sturgeon	

REVIEWER:

Surname: Yamaha	**Institution:** Nanae Freshwater Station, Field Science Center for Northern Biosphere, Hokkaido University.
Name: Etsuro	
Titles: PhD.	**E-mail:** eyamaha@fsc.hokudai.ac.jp
Please describe your professional relationship to the PhD student: No relation.	**Please describe your field of expertise:** Fish embryology, Fisheries Science

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Polyspermy is a strange phenomenon of fertilization embryologically. It has been believed that egg with plural number of sperm cleaved abnormally, and died during embryonic development in teleost. On the other hand, in sturgeon, it is well known that the eggs have many micropyles in their chorion and that polyspermy is common phenomenon in their fertilization. But, the details of the phenomenon and subsequent development of the embryos have been unclear. The author investigated the physiological polyspermy and development of subsequent embryos in detail, using variety of analyzes, such as flow-cytometry, electron-microscopy, microsatellite analysis and so on. Her results disclosed that polyspermy frequently occurs, depending on the concentration of sperm, and on distribution area of micropyles, resulting in atypical cleavages with 3-, 5-, 6-, 7-, 9-, 10 blastomeres at 2-4 cell stage. More interestingly, these embryos with atypical cleavages developed normally for four months, and some resultant individuals have both cells developed from blastomeres with parents' karyogamy and from those with additional paternal genome. These results contribute basic embryology in sturgeon. In addition, based on these results, author induced viable individuals with "three" genomic parents, successfully, according to her unique plan. This point shows highly originality and indicates the unique shift of thinking. If this induced mosaic with three genomic parents bear gametes only from blastomeres with additional paternal nucleus, her result will support for sturgeon breeding as important methods for applied biology of fisheries science. In this point, this work has high originality. Actually, it was very interesting reading this PhD thesis scientifically. Therefore, I think that her studies is worth while PhD thesis in your university.

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

There is no problem in structuring, comprehensibility, logicality and originality of this PhD thesis. I believe that author was required with high manipulation skill on fertilized eggs, and took many times for cultivation and rearing of many experimental fish. I think the publications have high quality.

I understand that all events could not be studied under limited time. However, I think following phenomenon are worth while analyzing, hereafter:

1) Degeneration of multi-sperm pronuclei during the first cell cycle. As some male pronuclei disappear from the egg cytoplasm during the first cell cycle, there must be certain mechanism to eliminate additional sperm nuclei. This must be the fundamental mechanism for monospermy in sturgeon.
2) Are all eggs fertilized with multi-sperm develop normal individuals? I think certain number of polyspermic eggs could be destined to lethal, because of aneuploidy and/or anuclear blastomeres.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Some comments are checked in the text. Please use it for your revision. And I have some queries and questions in following point:

1) In chapter 1, author mentioned that androgenesis can be used for production of inbred strains and clones, sex regulation, genetic maps, for analyzing the influence of mitochondrial DNA on the adaptive and economically important signs. But, in androgenetic individuals, not only mitochondrial DNA but also maternal egg cytoplasm, produced by maternal genomes during oogenesis, affect on the paternal genome. In this context, androgenesis can be used for nucleo-cytoplasmic interaction, too. What kinds of signs are induced in the cells with only paternal genome?
2) In chapter 1.5, author described that polyploid organisms are famous by rapid growth and disease resistance. This is not general in teleost. Triploid individuals induced in teleost do not always show rapid growth. Female triploid grows bigger occasionally in size over maturation period than diploid individuals, because their sterility. Sterility concerned with ploidy induce occasionally rapid growth and bigger size.
3) In genetics, a mosaic means the presence of two different genotypes in an individual which developed from a single fertilized egg. As a result, the individual has two or more genetically different cell lines derived from a single zygote. On the other side, chimaera is a single organism composed of cells with distinct genotypes. In animals, this means an individual derived from two or more zygotes. A zygote is a cell formed by a fertilization event between two gametes. Your fish derived from eggs fertilized with multiple-sperm, and composed of two or more different genotypes in an individual. But, they derived from neither a single zygote, nor two or more zygotes. Then, what do you think which name is better of your fish, mosaic or chimera? What is the reason?
4) Have your ever use the eggs from natural female in your experiments? I think, the author maybe used eggs only from female reared in artificial condition for many years as the materials. As the adhesion of eggs from such female fish is very weak, the removal of adhesion is easier than those from captured female in natural. Were there any report about polyspermy experiments, using eggs from natural female? Is there a possibility that polyspermy occurs easily in the eggs from reared female, like adhesion? Do the epigenetic effects during oogenesis cause polyspermy? What do you think about this idea?
5) In the case of tetraploid male in crucian carp, they occasionally produce diploid sperm and viable

diploid androgenetic offspring are obtained. In sturgeon, were there any reports that viable triploid or diploid individuals were generated from tetraploid or hexaploid parents, respectively, by artificial gynogensis or androgenesis? Are tetraploid and hexaploid sturgeons genetically diploid species?

6) Hybridogenesis, namely elimination of paternal genome and premiotic endomitosis of maternal genome during gametogenesis, have been reported in several hybrid fish, such as Poeciliopsis and Hexagrammos species. Are there any reports about hybridogenesis in several hybrid between sturgeon species? Do you think hybridogenesis occur in sturgeon hybrid occasionally?

7) The author discussed about haploid-derived clonal gametes. Premiotic endomitosis, namely duplication of haploid genome in germline cells before meiosis, should be required for gamete differentiation from haploid primordial germ cells. In this process, lethal mutations in haploid genome are also duplicated and prevents gamete differentiation. In order to avoid this harmful effect of lethal mutation, I think many number of haploid primordial germ cells, namely many number of multiple-sperm mosaics, should be prepared. In addition, there are some possibilities that diploid or triploid gamete differentiate from nucleus from tetraploid or hexaploid sperm, and that hybridogenetic maternal gametes from multiple-sperm mosaic? What do you think about this? Do you have any good solutions about this?

8) If the premiotic endomitosis occurs in haploid primordial germ cells, it is a possible story that it also occurs in those with hybrid zygote in multiple-sperm mosaics. What do you think so? Why does not the premiotic endomitosis occurs in primordial germ cells with hybrid zygote? If occur, do you have another application of multiple-sperm mosaics in biotechnology of fisheries science?

9) In sturgeon, author's idea, gamete production form blastomere with additional nucleus in multiple-sperm mosaic, is very interesting in new biotechnology. But, this idea could be only appliable to fish like sturgeon with special way of fertilization, polyspermy. How will this idea be applied to other fish species with mono-spermic fertilization through micropyle? Please let me know the idea.

FINAL RECOMMENDATION

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- [ ] **PhD Thesis can not be recommended for defence**

Oct. 6, 2018. Hakodate
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Date and place

山羽悦郎
Etsuro Yamaha
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Name and signature