

**Confidential**

**Review of USB FFPW PhD Thesis**

<b>First name(s), surname, titles of the PhD student:</b> Ievgen Lebeda, M.Sc.	
<b>First name(s), surname, titles of supervisor:</b> Prof. Dipl.-Ing. Martin Flajšhans, Dr.rer.agr.	
<b>Title of PhD thesis:</b> Optimization of chromosomal manipulations in Acipenserids	
<b>REVIEWER:</b>	
<b>Surname:</b> Kalous	<b>Institution:</b> Česká zemědělská univerzita v Praze Kamýcká 961/129 Praha 6 - Suchdol
<b>Name:</b> Lukáš	
<b>Titles:</b> Assoc. Prof. Dipl.-Ing., Ph.D.	<b>E-mail:</b> kalous@af.czu.cz
<b>Please describe your professional relationship to the PhD student:</b> None	<b>Please describe your field of expertise:</b> subjects: aquaculture, ichthyology, non-native species; methods: cytogenetics, molecular genetics, morphology, taxonomy

**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**):

Presented PhD thesis is consistent work of high scientific importance especially in applied science. I appreciate practical approach, which open up and clarify available scattered information regarding chromosomal manipulation in sturgeons. Undoubtedly the work shifts the knowledge in international context and has high relevance for aquaculture and conservation of Acipenserids. All of that also corresponds to the originality of the work.

**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**):

To evaluate the overall impression of the PhD thesis I am satisfied. There are few grammatical mistakes and the text reads nicely. The structuring of the main text is well done as well as comprehensibility and logicity of the chapters and their ordering. I found the selected

approaches to solve the objectives as original. The results correspond to objectives of the PhD thesis. Selected publications and manuscripts are consistent with objectives.

### **OVERALL COMMENTARY ON THE PhD THESIS**

**Please write comments in extent of 1-2 pages:**

The PhD thesis composes of an introduction, which is well written overview of chromosome manipulation, which leads smoothly to the definition of aims of the study. Four aims of the thesis are clearly defined and they are connected to five consequent chapters. The chapter 2 is the article in the IF journal *Aquaculture international* with the title: "Optimization of sperm irradiation protocol for induced gynogenesis in Siberian sturgeon, *Acipenser baerii*" this article came through tough reviewing process and does not need to be further evaluated. The chapter 3 is an article in press in the IF journal *Czech Journal of Animal Science* titled: "Chemical induction of haploid gynogenesis in sterlet, *Acipenser ruthenus*". Also in this article the reviewing process was completed and I do not have further comments. The chapter 4 is a manuscript titled: "Influence of photoreactivation on gynogenesis induction in sterlet, *Acipenser ruthenus*" it brings required technical conditions came to the clear conclusion that should be accepted in practice. The chapter 4 is manuscript titled: "Use of flow cytometry to assess success rate of gynogenesis induction and separate nongynogenetic progeny of sturgeon". It is well-prepared trial which proof the possibility of usage of flow cytometry to measure the success of the chromosome manipulation treatment. The chapter 5 is manuscript in form of short communication titled: "Optimization of tetraploidization protocol in sterlet, *Acipenser ruthenus*" it is again technical paper based on well-prepared trial bringing important information for practical usage of tetraploidization process induced by heat shock. This work is consistent, technically based and strongly affects the practical application of chromosomal manipulations in sturgeon aquaculture.

It was difficult for me as the reviewer to handle particular specific and well-designed trials with the proper statistical approach. When I was stuck on some methodical approach presented in the thesis, I did not dare to call in question it during the second reading. I am either insufficiently familiar with the issue, or it is simply right. Since I do not want to be challenged as an evaluator, I am inclined to the second version. I consider it as job well done. I can imagine the work load in hatchery and the number of Eppendorf tubes with a variety of labels. I fully appreciate the amount of painstaking work.

At this point, let me ask you a few questions.

- 1) How's the state of manuscripts at the moment?
- 2) Can you describe how it could work with the caviar production in the future (process, price, market)?
- 3) Is there any record of natural gynogenesis in acipenserids? Can it be expected?

### **FINAL RECOMMENDATION**

**PhD Thesis can be recommended for defence**

Date and place: 19.6.2014, Praha

Name and signature: doc. Ing. Lukáš Kalous, Ph.D.



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**Review of USB FFPW PhD Thesis**

<b>First name(s), surname, titles of the PhD student:</b> Ievgen Lebeda, M.Sc.	<b>First name(s), surname, titles of supervisor:</b> Prof. Dipl.-Ing. Martin Flajšhans, Dr.rer.agr.
<b>Title of PhD thesis:</b> Optimization of chromosomal manipulations in Acipenserids	

**REVIEWER:**

<b>Surname:</b> Fopp-Bayat	<b>Institution:</b> University of Warmia and Mazury in Olsztyn Oczapowskiego 2 str. 10-719 Olsztyn Poland
<b>Name:</b> Dorota	
<b>Titles:</b> Dr. hab. Ing.	<b>E-mail:</b> foppik@uwm.edu.pl
<b>Please describe your professional relationship to the PhD student:</b> Supervisor of PhD candidate during his 3-months foreign stay in the molecular laboratory, Department of Ichthyology, UWM in Olsztyn, Poland	<b>Please describe your field of expertise:</b> Sturgeon aquaculture, sturgeon reproduction, genome manipulations, molecular analysis, genetic identification

**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 chromosomal manipulations study are very important in aquaculture, especially in sturgeon fish that are producers of black caviar. The chromosomal manipulations, particularly gynogenesis is under a spotlight as a way to change ratio of females to males in progeny. However the induction of gynogenesis in sturgeon fish, based on UV-irradiated sperm, is not a new approach – the application on chemical agents, that damage sperm DNA, is the new ambitious challenge, that can be applied in large-scale production of gynogenetic offspring of sturgeons. Very significant part of dissertation is the application of flow cytometry during identification of putative gynogenetic offspring obtained during induction of gynogenesis using UV-irradiated heterologous sperm with different ploidy level. An important contribution of the thesis was the investigation of photoreactivation in *Acipenser ruthenus* sperm during induction of meiotic gynogenesis.

The last important contribution of the thesis, related to optimization of protocol for tetraploidization in sterlet, represents a great interest due to possible application of optimized parameters for production of highly homozygous gynogenetic progeny.

The results obtained in presented dissertation could have significant impact to basic science and also to practical utilization in sturgeon aquaculture.

*Dr*



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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, 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**):

Presented PhD thesis deals with topical study of optimization of chromosomal manipulation in Siberian sturgeon *Acipenser baeri* and sterlet *Acipenser ruthenus*. The doctoral dissertation contains 112 pages (including two published manuscripts, one manuscript under review, and two unpublished works) and consist of the 7 chapters. PhD candidate Ievgen Lebeda proceeds his scientific ability to elaborate PhD thesis. He has applied adequate research tools and methods to solve experimental objectives of optimization of selected chromosomal manipulations in Siberian sturgeon and sterlet. The four aims of the doctoral thesis were solved. Conclusion of proposed dissertation is comprehensible derived from results based on experimental work. The thesis is elaborated, well-arranged and it does not contain significant mistakes. In proposed doctoral thesis PhD candidate clearly demonstrated ability of independent scientific work.

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### **OVERALL COMMENTARY ON THE PhD THESIS**

**Please write comments in extent of 1-2 pages:**

#### **Review of PhD thesis**

**Title: Optimization of chromosomal manipulations in Acipenserids**

**Applicant: M.Sc. Ievgen Lebeda**

Presented PhD thesis deals with topical study of optimization of chromosomal manipulation in Siberian sturgeon *Acipenser baeri* and sterlet *Acipenser ruthenus*. The doctoral dissertation contains 112 pages (including two published manuscripts, one manuscript under review, and two unpublished works) and consist of the 7 chapters.

**Chapter 1.** General introduction (from page 6 to page 31) – describes application of genomic manipulation in fish with emphasizes on sturgeons including characteristics of UV irradiation parameters protocols for induction of gynogenesis in different fish species.



**Chapter 2.** Optimization of sperm irradiation protocol for induced gynogenesis in Siberian sturgeon, *Acipenser baeri* (from page 32 to page 44) – describes the procedures of optimization the UV irradiation of sperm in Siberian sturgeon. This parts of dissertation was published in Aquaculture International journal in 2014.

**Chapter 3.** Chemical induction of gynogenesis in sterlet *Acipenser ruthenus* (from page 45 to page 70) includes the results of induction gynogenesis experiments with application of four chemical agents: dimethyl sulfate (DMS), ethidium bromide (EB), psoralen (PS) and 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT). This chapter was submitted to Czech Journal of Animal Science and is in press.

**Chapter 4.** Influence of photoreactivation on gynogenesis induction in sterlet, *Acipenser ruthenus* (from page 71 to page 80) deals with the study of photoreactivation influence on induction of meiotic gynogenesis in sterlet. This chapter was submitted to the Aquaculture Research journal and is under review.

**Chapter 5.** Use of flow cytometry to assess success rate of interspecific gynogenesis induction and separate nongynogenetic progeny of sturgeon (from page 81 to page 90) includes the description of the application of the flow cytometry technique in verification of ploidy in genome manipulated progeny that may also be reliable for separation nongynogenetic progeny of Siberian sturgeon and Siberian sturgeon x sterlet hybrids.

**Chapter 6.** Optimization of tetraploidization protocol in sterlet *Acipenser ruthenus* (from page 91 to page 97) deals with the optimization of protocol for tetraploidization in sterlet that succeeded with production of 34% of tetraploid larvae.

**Chapter 7.** General Discussion, English and Czech Summary, Acknowledgments, List of publications, Training and Supervision Plan during study and Curriculum Vite (from page 98 to page 112) summarize the all achievements and describes the profile of PhD candidate.

The thesis brings topics that are currently very important in reproductive biology of sturgeons and its aquaculture. However the genome manipulation in sturgeon fishes based on UV-irradiated sperm is not a new approach – the application on chemical agents, that damage sperm DNA, is the new research challenge in Acipenserids, that can be applied in large-scale production of gynogenetic offspring. Very significant part of dissertation is the application of flow cytometry during identification of putative gynogenetic offspring obtained during induction of gynogenesis using UV-irradiated heterologous sperm with different ploidy level.

An important contribution of the thesis was the investigation of photoreactivation in *Acipenser ruthenus* sperm during induction of meiotic gynogenesis. Although this parts of doctoral dissertation is very important and interesting it contains the basic failure - the lack of molecular verification of gynogenetic offspring. In experiments that the homologous sperm was applied during induction of gynogenesis the molecular verification should be applied, especially when the spermatozoa is subjected on low UV-irradiation. Only molecular markers (e.g. microsatellites) allows a proper verification of effectiveness of chromosomal manipulations when the homologous sperm was used. In proposed approach the molecular analysis could be omitted if the experiment had been conducted with application of heterologous sperm.

The last important contribution of the thesis, related to optimization of protocol for tetraploidization in sterlet, represents a great interest due to possible application of



optimized parameters for production of highly homozygous gynogenetic progeny. The results obtained in presented dissertation could have significant impact to basic science and also to practical utilization in sturgeon aquaculture.

**Questions to be answered:**

What differences are in sperm DNA inactivation using UV irradiation and chemical agents ?  
What changes in the DNA structure are characteristic of these two techniques of sperm inactivation ?

**Conclusion**

PhD candidate levgen Lebeda proceeds his scientific ability to elaborate PhD thesis. He has applied adequate research tools and methods to solve experimental objectives of optimization of selected chromosomal manipulations in Siberian sturgeon and sterlet. Conclusion of proposed dissertation is comprehensible derived from results based on experimental work. The thesis is elaborated, well-arranged and it does not contains significant mistakes.

PhD candidate levgen Lebeda has presented his research results of various conferences that were accepted by International Scientific Community. In proposed doctoral thesis PhD candidate clearly demonstrated ability of independent scientific work. Proposed thesis fulfill all-important criteria and thus I recommend proposed thesis to state PhD defense. After successful tract and conclusion of the defense, I recommend further to give an academic title PhD.

Dr hab. inž. Dorota Fopp-Bayat, prof. UWM

**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

*Olsatym, 16.06.2014*

Date and place

*DOROTA FOPP-BAYAT*

*Dorota Fopp-Bayat*

Name and signature