



**University of South Bohemia in České Budějovice  
Faculty of Fisheries and Protection of Waters  
Institute of Aquaculture**

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**Confidential**

**Review of USB FFPW PhD Thesis**

Surname of the PhD student: Anna Shaliutina, MSc.	Name of supervisor: Prof. Dipl.- Ing. Otomar Linhart, DSc.
Title of PhD thesis: Fish sperm motility parameters and total proteins in seminal plasma during <i>in vivo</i> and <i>in vitro</i> storage	
<b>REVIEWER:</b>	
Surname: Inaba	Institution: University of Tsukuba, Shimoda Marine Research Center
Name: Kazuo	
Titles: Prof., Ph.D.	E-mail: inaba@kurofune.shimoda.tsukuba.ac.jp
Please describe your professional relationship to the PhD student: host researcher for her temporal study in Japan, 2012.	Please describe your field of expertise: molecular and cell biology, reproductive biology (sperm motility; cilia and flagella)

**QUESTIONNAIRE**

***Originality, scientific importance, prospects of the PhD thesis and benefits for basic or applied research***

Evaluate its competitiveness in the international context and compare its level with the current state of the art in the field:

The thesis describes the effect of preservation of semen on the motility and other physiology of sperm functions. It is based on basic biological aspects of sperm but potentially very important in applied fields such as fisheries sciences. The papers already published would contain basic and very important context for the development of suitable method for semen preservation. The works are scientifically and internationally valuable and the level is high in the relevant scientific field.

***Preparation of the PhD thesis, targets of the work and deliverables***

Evaluate the overall level of preparation of the PhD thesis and the originality of the selected approaches; evaluate publications and whether the targets set in the PhD thesis correspond with the declared purpose of the thesis:

The organization of this PhD thesis is well considered and well achieved. Especially the evaluation of cryopreservation by several parameters of sperm motility and by proteomic approach is new and could be an internationally leading research. I am not sure the rule of PhD thesis in the University of South Bohemia, but is it general that published papers appear as they appear in each journal? Chapters 2-6 are all PDFs of published papers in specific format of each journal. I recommend to revise the PhD as to write Chapter 2-6, without pasting journal PDF (just paste text, figures and tables).



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

**Please write comments:**

The thesis is written in accordance with its four objectives:

1. To evaluate sperm production characteristics and spermatozoa motility parameters in teleostean and chondrosteian species during *in vivo* storage.
2. To describe the effects of *in vivo* storage on protein composition of seminal plasma in models of chondrosteian and teleostean fish species.
3. To examine the effect of *in vitro* storage on parameters of sperm motility, DNA damage and oxidative stress in sperm of chondrosteian fish species.
4. To investigate if different *in vivo* storage time could influence sperm cryoresistance and post-thaw fertilizing ability in chondrosteian fish species.

All the objectives have been properly achieved in this thesis. In particular, it provides much important data, including motility parameters, protein composition of seminal plasma, DNA damage, oxidative stress during *in vivo* storages. All the data would become an important knowledge for studying the role of seminal plasma and for developing an appropriate procedure for storage of semen of Chondrosteian species.

Although these data are very important, I recommend the candidate to mention a little more about:

1. Possible mechanism on how the parameters that change during storage affect on sperm physiology.
2. A proposal for the method of better preservation

As for 1, for example, the relationship between oxidative stress and sperm motility may be more discussed. The candidate has examined the changes in proteins at 2D-PAGE level. It would be better to discuss by giving a specific protein that is potentially susceptible for oxidative stress or is important for sperm motility. As for 2, this seems to be a future issue. However, the candidate may give a proposal on what compounds are effective to prolong the viability, DNA normality and sperm motility as additive during storage. Some are appearing in the "General Discussion" but the point of view above may be further included.

Nonetheless, overall the context of this thesis is well organized and well written. The aim is clear and the research outcome in publication is substantial. The thesis deserves defence of PhD thesis.

### **FINAL RECOMMENDATION**

- can be recommended for defence of PhD Thesis
- PhD Thesis can be recommended with reservations for defence
- PhD Thesis can not be recommended for defence

...June 21, 2013, Shimoda, Japan...  
 Date and place

...Kazuo Inaba.....  
 Name and signature



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**

<b>First name(s), surname, titles of the PhD student:</b> Anna Shaliutina, MSc.	<b>First name(s), surname, titles of supervisor:</b> Prof. Dipl.- Ing. Otomar Linhart, DSc.
<b>Title of PhD thesis:</b> Fish sperm motility parameters and total proteins in seminal plasma during <i>in vivo</i> and <i>in vitro</i> storage	
<b>REVIEWER:</b>	
<b>Surname:</b> Cierieszko	<b>Institution:</b> Polish Academy of Science
<b>Name:</b> Andrzej	Institute of Animal Reproduction and Food Research Olsztyn, Poland
<b>Titles:</b> Professor	<b>E-mail:</b> a.cierieszko@pan.olsztyn.pl
<b>Please describe your professional relationship to the PhD student:</b> None	<b>Please describe your field of expertise:</b> Fish reproduction

## **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 results of the thesis have been published in peer-reviewed journals ranked very high by the ISI Web of Knowledge (Journal Citation Reports 2011), Reproduction in Domestic Animals (IF = 1.36) is ranked 37 (of 145) in the category „Veterinary Sciences”, Molecular Reproduction and Development (IF = 2.53) is ranked 13 (of 28) in the category „Reproductive Biology” , Animal Reproduction Science (IF = 1.75) is ranked 7 (out of 55) in the category „Agriculture, Dairy and Animal Science” and Aquaculture (IF = 2.04) is ranked 11 (of 50) in the category „Fisheries”. As such, the results of the thesis were subjected to a very strict peer-review process and thus were validated according to very rigorous standards of the scientific process. In my opinion, this thesis is well focused around the physiological events related to fish sperm motility and sperm proteome. For the first time, numerous novel sperm proteins have been identified and their presence have been linked to important physiological processes, such as dynamic of sperm production, seasonal effects and sperm fertilizing ability. This thesis also adds valuable information important for improvement of reproductive technologies, such as multiple milt collections, short-term storage, and cryopreservation. Results obtained are highly relevant for reproduction of sturgeon fish (sterlet) and teleost fish as well (perch). Overall, this thesis significantly contributes to the knowledge on male fish reproduction, both regarding basic (reproductive proteomics, spermiation, season) and applied (reproductive biotechnologies) research.



***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**):

The main part of the thesis is composed of five chapters which are reprints of published papers. Chapter 2 provides information concerning sperm production indices with multiple sperm collections in sterlet. Comparison of protein fractions in seminal plasma from multiple collections in sterlet is presented in chapter 3. Chapter 4 demonstrates, that season is an important factor responsible for variability in semen quality parameters, such as sperm and protein concentrations and semen volume. Moreover, 10 protein spots were significantly altered over the course of the reproductive season. Chapter 5 deals with changes in sperm quality parameters during short-term storage of sterlet semen. Parameters of DNA integrity and oxidative stress were used, which allowed complex evaluation of sperm damage. Chapter 6 presents information concerning semen parameters of sterlet as well as its cryoresistance in relation to sequential stripping. The scientific part of the thesis is preceded by the introductory chapter 1, in which the author adequately reviews the topics related to the quality of fish sperm, especially in relation to sperm motility, the problem of oxidative stress, and seminal proteins. In my opinion this part would benefit from brief information concerning proteomic methods and the results of proteomic studies of fish semen, since this is an important issue of the thesis. General discussion (chapter 7) follows the scientific part; the main achievements of the thesis are summarized and concluded. The thesis consists of four papers concerning sturgeon and only one concerning yellow perch, which resulted in quite an impressive amount of information. On the other hand, perhaps an alternative way to compose the thesis would be to focus on sturgeon papers only. The language of the thesis is clear. My concerns (listed below) are of minor importance and can be easily corrected.

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**OVERALL COMMENTARY ON THE PhD THESIS**



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

As I mentioned above, this thesis covers a set of original topics related to sperm motility and seminal proteins in teleost and sturgeon fish. The thesis is written in a clear manner and the results of the thesis were already published in top peer-reviewed journals. Basic and applied aspects of fish reproduction are well balanced in the thesis, from basic studies of seminal proteins to testing specific sperm quality indices in relation to short-term storage and cryopreservation.

It needs to be mentioned, that Ms. Anna Shalutina, in addition to the papers included in the thesis, is the coauthor of 7 papers, 4 of which are published and the rest are under review. This number is notable and confirms the excellence of her research and her competence in scientific work.

In conclusion, my overall grade of the thesis is excellent and I strongly recommend the thesis for the defense of PhD thesis.

Minor corrections that can be used to improve the text are listed below.

P6, P61 Czech Summary?

P10/1<sup>st</sup> para I would mention activation mode of salmonid fish sperm (dependence on potassium concentration in order to make this paragraph complete).

P10/2<sup>nd</sup> para there is extensive literature concerning season-dependant changes in sperm concentrations in vivo.

P11/2<sup>nd</sup> PUFA was introduced on the previous page;

P11/3<sup>rd</sup> para/L3 I do not think that low levels of antioxidants cause oxidative damage

P12 please add a sentence regarding urine, because it is a major factor influencing measured values of osmolality.

P13 Papers of Perez-Pe et al., and Marti et al., concern ram semen. It should be mentioned in the text or this part need to be modified through focusing on fish.

Table 1, Ubiquitins are not proteolytic enzymes; no information on functions of phosphatases are provided; *C. carpio*

P14, last para, a reference for creatine kinase is needed.

P64/3<sup>rd</sup> para Paper of Zhao et al. concerns nematode sperm.

P66 a title "Cryopreservation..." is only partially relevant to the context of this paragraph.

P66 there is a mixture of summaries and true conclusions in this part (for example #1 and #2)

## **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**

10 April 2013, Olsztyn  
Date and place

Andrzej Cierieszko...  
Name and signature