

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

Branišovská 1645/31a, 370 05 České Budějovice, Czech Republic  
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**Confidential**

**Review of USB FFPW PhD Thesis**

Name of the PhD student: Li Ping	Name of supervisor: Prof. Dipl. – Ing. Otomar Linhart, Dr.Sc.
Title of PhD thesis: Use common carp ( <i>Cyprinus carpio</i> L.) sperm to study the cryoinjuries by cryopreservation	

**REVIEWER:**

Surname: <b>Ciereszko</b>	Institution: Institute of Animal Reproduction and Food Research, Polish Academy of Sciences
Name: Andrzej	
Titles: Prof.	E-mail: <a href="mailto:a.ciereszko@pan.olsztyn.pl">a.ciereszko@pan.olsztyn.pl</a>
Please describe your professional relationship to the PhD student: I am co-author of one paper with P. Li (Psenicka et al. 2011, J. Appl. Ichthyol. 27, 678–682.	Please describe your field of expertise: <b>Fish reproduction</b>

**QUESTIONNAIRE**

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

Results of the thesis have been published in peer-reviewed journals ranked very high by the ISI Web of Knowledge (Journal Citation Reports 2009). Biology of Reproduction (IF = 3.30) is ranked 5th (of 26) in the category „Reproductive Biology“, Theriogenology (IF = 2.07) is ranked 12 (of 142) in the category „Veterinary Sciences“ and Fish Physiology and Biochemistry (IF = 1.23) is ranked 20 (of 42) 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, the most important scientific achievements of the thesis are:

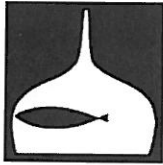
- -proteomic analysis of fresh and cryopreserved carp sperm;
- evaluation of effects of cryopreservation on antioxidants of carp semen;
- isolation of fraction of cryopreserved carp sperm of superior quality using Percoll gradient.

All of these issues are new and original, and were among the top achievements in the field of fish reproduction during last years. Overall, the scientific importance of the thesis is very high, both for basic research (regarding knowledge on the physiological and biochemical aspects of cryopreservation of fish semen) and applied research (concerning improvement of cryopreservation technology of fish semen). The latter is important for fish aquaculture (improvements of breeding methods) and for the preservation of biodiversity of domesticated and wild fish species.

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

The main part of the thesis is composed of five chapters containing scientific results. Chapter 2 provides a review of sperm proteins and describes (the use of these) proteins as quality markers during cryopreservation. This information was especially useful for the discussion of the results presented in chapter 3, concerning proteomic changes due to cryopreservation of carp semen. Chapter 4 presents data on changes in antioxidants after cryopreservation and chapter 5 deals with Percoll gradient separation of cryopreserved semen.

The scientific part of the thesis is preceded by the introductory chapter 1, in which the author adequately reviews topics related to the technology of cryopreservation and cryoinjuries. Chapter 7 summarizes the main achievements of the thesis. All together, all parts of the thesis are very well connected and the subjects of the thesis are well organized. The language of the thesis is very clear and allows the readers to quickly grasp the main ideas of the work. My only concern is chapter 5, presenting the results of phosphorylation status of carp proteins. These results have not yet been published and I could not evaluate this part due to very limited information included in this chapter. The thesis would also benefit from a concluding paragraph. These concerns are of minor importance and can be easily corrected.



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**Please write comments:**

As I mentioned above, this thesis covers set of original topics linked together in a logical way. The thesis is written very clearly and the results of the thesis were already published in top peer-reviewed journal. Basic and applied aspects of fish reproduction are well balanced in the thesis, from basic proteomic studies of carp semen to elaboration of the method of isolation of good quality spermatozoa from cryopreserved semen. It also needs to be mentioned, that Ms. Ping Li, besides papers included in the thesis, is coauthor of 29 peer-reviewed papers. This number is impressive and confirms excellence of her research and her competence to scientific work.

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

**OVERALL COMMENTARY ON THE PhD THESIS**

**FINAL RECOMMENDATION**

can be recommended for defence of PhD Thesis

can be recommended with reservations for defence of PhD Thesis

can not be recommended for defence of PhD Thesis

27 May 2011, Olsztyn, Poland.....  
Date and place

Andrzej Ciereszko  
Surname and signature



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**Please write comments:**

In chapter 1, general aspects and facing problem of cryopreservation of fish spermatozoa are extensively and adequately described. In chapter 2, general aspect of the molecular components of spermatozoa and its situation in fish spermatozoa are well summarized. In chapter 3, the effects of cryopreservation on the protein components of fish spermatozoa are described based on her research with biochemical approach. In particular the use of proteomic technique in identification of protein components that are affected by cryopreservation is worth being admired. In chapter 4, she approaches to elucidate the effects of cryopreservation on spermphysiology, focusing on sperm motility. Chapter 5 and 6 are quite preliminary, but I would like to anticipate her to complete these works.

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 deserve defence of PhD thesis.

**Review of USB FFPW PhD Thesis**

Surname of the PhD student: M.Sc. Li Ping	Name of supervisor: Prof. Dipl. – Ing. Otomar Linhart, Dr.Sc.
Title of PhD thesis: Use common carp (Cyprinus Carpio L.) sperm to study the cryoinjuries by cryopreservation	

**REVIEWER:**

Surname: Inaba	Institution: University of Tsukuba, Shimoda Marine Research Center
Name: Kazuo	
Titles: Prof., Ph.D.	E-mail: <a href="mailto:inaba@kurofune.shimoda.tsukuba.ac.jp">inaba@kurofune.shimoda.tsukuba.ac.jp</a>
Please describe your professional relationship to the PhD student: host researcher for her temporal study in Japan, 2008.	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:

Evaluation and assessment of cryopreservation of fish spermatozoa are of great value, especially in that the author gives priority on molecular aspects. The thesis is based on basic biological aspects but potentially very important in applied fields such as fisheries sciences. The context can be scientifically and internationally valuable and unpublished one. 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 construction of this PhD thesis is well considered and well achieved. Especially the evaluation of cryopreservation by proteomic approach is new and could be one of the pioneer work that would be the model paper for future following works using different species of fish. One point is: I wonder if it is necessary to include the part of Chapter 5 and 6 into her PhD thesis. These are very preliminary. Chapters 1-4 themselves are excellent and appear enough staff for defending PhD thesis.

**OVERALL COMMENTARY ON THE PhD THESIS**



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***FINAL RECOMMENDATION***

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

June 15, 2011, Shimoda, JAPAN  
Date and place

Kazuo Inaba  
Surname and signature