



**Confidential**

**Supervisor's Review of USB RIFCH PhD Thesis**

Surname of the PhD student: <b>Yevhen Horokhovatskyi</b>	Name of supervisor: <b>Dzyuba Borys</b>
Title of PhD thesis: Applied aspects of fish sperm cryopreservation	

**OVERALL COMMENTARY ON THE PhD THESIS**

Yevhen Horokhovatskyi joined Laboratory of Reproductive Physiology as a PhD student in 2014 after receiving of MSc diploma in Applied Physics from the Department of Biological and Medical Physics, V. N. Karazin Kharkiv National University, Kharkiv (Ukraine).

His level and area of education allowed him a quite quick mastering of basic video microscopy and techniques of controlled and non-controlled freezing of biological objects required for his Ph.D. studies. In addition, Yevhen was actively studying biochemical methods necessary to assess cell lipid and protein composition, as well as cell separation methods which also contributed a lot into performance of his PhD project targeted to increase the fish sperm cryopreservation outcomes, and included among other the following issues: 1) elucidation of reasons for heterogeneity of fish sperm cryoresistance, 2) standardization of freezing protocols and 3) elaboration of methods allowing deeper understanding of biological objects' cryodamage.

The mentioned above issues are considered nowadays as an important ones for effective fish sperm cryobanking and this is quite well explained in introduction part of the thesis. The experiments performed during Yevhen's PhD study demonstrated that lipid composition in common carp sperm is associated with their cryoresistance (Chapter 2), the cryopreservation outcomes after application of non-controlled cooling devices can be compromised by inappropriate loadings of samples into devices (Chapter 3). Finally, the method for collection of populations of sturgeon sperm with high motility traits was developed and applied for the detection of changes in proteome in sperm which successively survived cryopreservation (Chapter 4). I would like to underline that this approach opens novel perspectives for clear evaluation of cryodamage in fish sperm. I am glad to note that Yevhen successfully completed his PhD thesis and his findings are useful for future progress in fish sperm cryobiology and could be applied in fish sperm cryobanking.

It is also worth to mention that Yevhen had useful internships in several prominent scientific laboratories such as Department of Gamete and Embryo Biology, Institute of Animal Reproduction



and Food Research, Polish Academy of Sciences, Olsztyn, Poland (Prof. Andrzej Ciereszko); Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden (Prof. Jane M Morrell), and Fish Physiology and Genomics Institute, Rennes, France (Prof. Catherine Labbe). In these laboratories Yevhen mastered contemporary techniques of proteomics and cell separation and was also involved in activity of conservation program on restoration of critically endangered European Atlantic sturgeon *Acipenser sturio* population.

Finally I want to recommend thesis of Yevhen Horokhovatskyi for defense and I am looking forward for our future cooperation.

### **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

30.04.2018, Vodnany  
Date and place

B. Dzyuba,   
surname and signature