

**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: M.Sc. Sergey Boryshpolets	Name of supervisor: Prof. Dipl.-Ing. Otomar Linhart, DSc.
Title of PhD thesis: <b>Energetic and motility of fish spermatozoa</b>	
<b>REVIEWER:</b>	
Surname: Ciereszko	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 S. Boryshpolets (Psenicka et al. 2011, J. Appl. Ichthyol. 27, 678–682.	Please describe your field of expertise: <b>Fish reproduction</b>

**QUESTIONNAIRE**

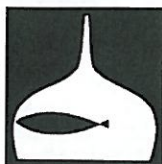
**Originality, scientific importance, prospects of the PhD thesis and benefits for basic or applied research**

The results of the thesis have been published in peer-reviewed journals ranked very high by the ISI Web of Knowledge (Journal Citation Reports 2009), Theriogenology (IF = 2.07) is ranked 12 (of 142) in the category „Veterinary Sciences“, Cryobiology (IF = 1.72) is ranked 33 (of 76) in the category „Biology“, Fish Physiology and Biochemistry (IF = 1.23) and Journal of Applied Ichthyology are ranked 20 and 24 (of 42) in the category „Fisheries“, respectively. 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 very well focused around the phenomenon of multiple activation of fish spermatozoa. For the first time, various aspects of sperm reactivation has been studied in a systematic way and published in a series of papers. In my opinion, the discovery of spontaneous sperm activation by cryopreservation is an important contribution to the knowledge on reproduction of fish male. Overall, the scientific importance of the thesis is very high, both for basic research (regarding knowledge on mechanisms of sperm activation in fish) and applied research (concerning improvement of fertilization and cryopreservation technology of fish semen).

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

The main part of the thesis is composed of four chapters which are reprints of published papers. Chapter 2 provides information concerning respiration and reactivation parameters of carp spermatozoa in relation to pre-spawning water temperature. Repetitive activation of Eurasian perch spermatozoa is presented in chapter 3. Chapter 4 demonstrates, that freezing-thawing is an important factor responsible for spontaneous activation of carp sperm motility. This phenomenon was tested for different fish species (chapter 5). The scientific part of the thesis is preceded by the introductory chapter 1, in which the author adequately reviews the topics related to energetics of fish sperm, especially in relation to motility. In my opinion this part would benefit from brief information concerning pre-spawning temperature and semen quality (this should also be mentioned in aims of the study), cryopreservation and motility, because these issues are central in the thesis. General discussion (chapter 6) follows the scientific part; the main achievements of the thesis are summarized and concluded. All together, all parts of the thesis are very well connected and subjects of the thesis are cohesive. The language of the thesis is clear. My concerns are of minor importance and can be easily corrected.

**OVERALL COMMENTARY ON THE PhD THESIS**



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**Please write comments:**

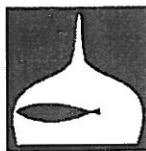
As I mentioned above, this thesis covers a set of original topics linked together in a logical way. The thesis is written in a clear manner and the results of the thesis were already published in top peer-reviewed journal. Basic and applied aspects of fish reproduction are well balanced in the thesis, from basic proteomic studies of motility reactivation carp semen to testing spontaneous activation of cryopreserved fish spermatozoa. It needs to be mentioned, that Sergey Boryshpolets, besides of papers included in the thesis, is coauthor of five peer-reviewed papers. This number is impressive and confirms the excellence of his research and his competence to scientific work. In conclusion, my overall grade of the thesis is excellent and I strongly recommend the thesis for the defence of 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**

...28 May 2011.....  
Date and place

Andrzej Ciereszko  
Surname and signature



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**Review of USB FFPW PhD Thesis**

Surname of the PhD student: M.Sc. Sergey Boryshpolets		Name of supervisor: Prof. Dipl.-Ing. Otomar Linhart, DSc.	
Title of PhD thesis: <b>Energetic and motility of fish spermatozoa</b>			
<b>REVIEWER:</b>			
Surname: Trippel		Institution: Fisheries and Oceans Canada, Biological Station/University of New Brunswick, Canada	
Name: Edward A.			
Titles: Prof.		E-mail: <a href="mailto:Edward.Trippel@dfo-mpo.gc.ca">Edward.Trippel@dfo-mpo.gc.ca</a>	
Please describe your professional relationship to the PhD student: No relationship		Please describe your field of expertise: Fish reproduction with specialization in male reproduction and fish spermatology	

**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 is highly original and of immediate scientific importance to the area of fish spermatology. It tackles some very intriguing issues of fish sperm in relation to their energetics, motility and cryopreservation. The approaches taken to elucidate these facets of fish sperm were very sound and right on the mark for what is needed in this field. The Ph.D. thesis and papers within will attract much attention and already is fuelling more exciting research to further our understanding of fish sperm dynamics. These original findings have great benefits for a number of freshwater fishes and are of import for basic and applied research. The selected species are or may be important for aquaculture and thus improving our understanding of sperm biology is essential for sound breeding programs that may utilize elite broodstock for breeding via cryopreservation.

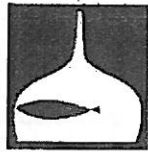
***Preparation of the PhD thesis, targets of the work and deliverables***

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 thesis is well prepared with a great General Introduction and Discussion and published papers, some in highly reputable journals. Thus, the thesis objectives and their execution are of a highest standard, with a few comments made below that can be used for consideration in future research.

Chapter 2: Prespawning water temperature affects sperm respiration and reactivation parameters in male carps

This innovative research demonstrated some interesting patterns in sperm respiration and their capacity to be reactivated in relation to 15 and 20 C fish holding temperatures. Reactivation was achieved by incubating initially activated sperm in media with osmotic pressure adjusted up to 300 mOsm\*kg<sup>-1</sup> by increasing K<sup>+</sup> concentration. The investigator hypothesized that a reduced water temperature during the pre-spawning period results in a slowing-down of spermiogenesis, manifesting in a decreased sperm concentration and differences in the respiration activity of spermatozoa. A few details in the methods, however, raise a few



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questions. Firstly, it is unclear why after sampling sperm from fish of the two temperature groups why an intermediate holding temperature (for not more than one hour) of 18 C was chosen. It would have more sense to maintain them at each of their respective temperatures, i.e., 15 and 20 C. Also, the room temperature and temperature of the slides used in the sperm motility analyses are not provided making it difficult to judge if any other short-term temperature exposure could have biased the results. Sperm motility analyses were only performed on samples for which >90% of sperm were swimming, thus there is potential bias introduced by this, for example if 15 C had a greater number of slides with slow sperm motility than at 20 C and these data are not reflected in the thesis results. Temperature of the pond water for the second activation is not provided. In Results section, data are reported in a way that indicates a rise occurred. However, there is no trend through time data, it should have been reported that one was higher or lower but not in reference to time. Apart from these criticisms, Chapter 2 yields some very interesting data, in particular Fig. 6 showing the differing patterns of percent motility between the first and second sperm activation phase.

#### Chapter 3 Dynamics of ATP and movement in Eurasian perch sperm in conditions of decreasing osmolality

This piece of work does a great job at elucidating that a sample of semen is comprised of different populations of sperm which become activated at different time periods based on specific conditions. The dynamics of ATP concentration were measured in post activated perch sperm for the first time. This was done by creating stepwise reductions in the osmotic pressure (via drop in glucose concentration) of a semen sample. It was discovered that repeated motility periods were possible only if the first activation was achieved at >200 mOsm/kg. The declines in ATP associated with these activations were reported. This stepwise reduction provided an extended period of sperm motility that might help to increase the fertilization rate of eggs and be used in perch breeding. The Discussion in this Chapter is particularly elegant and brings out some queries that have not been addressed previously. For example, significance of the measurement of total ATP level in relation to the function of various parts of a sperm cell. The suggestions of further research in relation to sperm head swelling are very appealing.

#### Chapter 4: Freeze-thawing as the factor of spontaneous activation of spermatozoa motility in common carp (*Cyprinus carpio* L.)

Cryopreservation is a vital tool for aquaculture and storing of gametes of endangered species. Thus, investigations that further the modification of techniques in the area of cryogenics are very useful. In Chapter 4, excellent research reveals that 'spontaneous' sperm activation may occur during the freeze-thawing procedure of carp sperm which might reduce the potential fertilization potency of a sample of frozen sperm. In the methods, however, it was noted that there existed no replication of straws in the experimental design, rather sperm were withdrawn from the same straw of each treatment, there were 3 straws but each was designed for a different test, there should have been 9 straws in total, 3 replicates for each test. This replication would have helped in the scientific validity of the reported results. It is not clear why two solutions were used for sperm activation. This Chapter nicely shows that fertilizing ability of sperm remains stable within a 10 min post-thaw storage period. It is speculated that the capacity for motility is retained as there still remains the potential for ATP distribution among undamaged and cells damaged by the freeze-thaw process. In conclusion, despite spontaneous motility, the freeze-thawing procedure does not impact fertility.

#### Chapter 5: Spontaneous activation of spermatozoa motility by routine freeze-thawing in different fish species

Further to Chapter 4, research is conducted on the basic biology of sperm during the freeze-thawing period for two groups of freshwater fish, one that requires changes in osmotic potential to stimulate movement (carp, pike and perch) and the other that requires changes in environmental ionic composition (effect of  $K^+$ ) (sturgeons and salmon). The loss of motility during the freeze-thawing process is well known and this work undertakes a better understanding of this process for possible improvement of cryopreservation procedures. The mechanism behind the spontaneous activation is purported to be the water thawing that was once frozen during the cooling of cell suspension. By studying a large number of fish species it was anticipated that a good understanding of the process would be obtained. It was shown that spontaneous motility occurred in 3 of the 6 species. In species without this process, sperm motility was monitored via the use of an activation medium. In the Results, there was a misuse of statistics in terms of non-overlap of 95% confidence intervals as a valid test for statistically significant differences.

Trippel, E. A., and J. J. Hubert. 1990. Common statistical errors in fishery research, p. 93-102. In J. Hunter (ed.) *Writing for Fishery Journals*. Am. Fish. Soc., Bethesda, MD.



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Please write comments:

**Preparation of Ph.D. Thesis (cont'd)** The process this occurs is thought to be that during cooling the cells entered hypertonic conditions, resulting from water freezing out. Then during thawing, the cells entered conditions of declining osmotic pressure, which happens during water crystal melting. Astute discussion was made of the sperm motility patterns for starlet in which increases of mean velocity was reported of freeze-thaw sperm perhaps due to disappearance of slow moving sperm that are only observed in fresh sperm samples.

**My overall judgement of the thesis is excellent, a job well done, Congratulations!**

### **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

.....June 22, 2011 .....  
Date and place

...Edward Trippel.....  
Surname and signature