Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice
Czech Republic

Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Deepali Rahi, Ph.D.	**First name(s), surname, titles of supervisor:** M.Sc. Viktoriya Dzyuba, Ph.D.
Title of PhD thesis: Fish sperm respiration: species specificity and effect of environmental temperature	

REVIEWER:

Surname: Perez	**Institution**: Polytechnic University of Valencia Spain
Name: Luz	
Titles: Full Prof.	**E-mail**: mlpereig@dca.upv.es
Please describe your professional relationship to the PhD student: none	**Please describe your field of expertise:** Fish reproduction, sperm physiology

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 PhD thesis comprises 3 works, and two of them have been already published in the international journals *Journal of Fish Biology*, and *Biology*, what supports its competitiveness in the international context. The level of the PhD thesis is similar to other works published in the same area, making an important contribution in the field of fish sperm bioenergetics. For that reason, probably these works will be very cited in future studies. The PhD includes a study on the burbot sperm, which is the first on the determination of the dominant energy-suplying pathway that contributes to the energy production for sperm motility at low and high temperatures. Also, it has been determined for the first time that sturgeon spermatozoa have the capacity for reactivation after termination of the first motility period. Also, it has been concluded that the oxidative phosphorylation in the fish studied (sturgeons and burbot) is actively participating in the energy supply of spermatozoa during quiescent and motile periods, although a major part of this energy is produced at the quiescent state. This knowledge is new and makes a valuable contribution to fish spermatology.

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, logicality 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 PhD contains 5 chapters; the first is an introduction, 3 are publications already published or in preparation, 5th includes is a general discussion of the publications, as well as acknowledgements and the CV of the student. The Thesis is well structured, in a logical way. The document seems elaborated with accuracy, without typological or other types of mistakes. The approach to study energy pathways by using selective inhibitors of the enzymes involved in glycolysis, fatty acids oxidation and glycolysis seems appropriate. Also, measurement of sperm oxygen consumption gives important information of fish bioenergetics. Regarding the objectives of the thesis:

1. To study the relative contribution of different bioenergetic pathways involved in energy supply in spermatozoa of Siberian sturgeon (*Acipenser baerii*) at motile and immotile state. This objective has been accomplished with a publication in *Journal of Fish Biology*.

2. To study the energy supplying pathways in an under-ice spawning fish burbot and adaptation of sperm mitochondrial activity to the maximum critical temperature. This objective has been accomplished with a publication in the journal *Biology*.

3. To study the possibility of sterlet sperm reactivation in terms of regaining motility and re-achieving fertilizing ability. 4. To evaluate the functioning of different bioenergetic pathways in quiescent, motile, and reactivated sterlet spermatozoa. This last two objectives have been accomplished in chapter 4, which has not yet been published.

OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

The introduction has a convenient extension, and introduces all the subjects that will be studied in the different chapters (Sperm motility duration, Energy suppying pathways, Role of mitochondrial respiration, Phosphocreatinine shuttle system and Sperm reactivation) It is well written and in a clear way, and does not contain unnecesary information

Chapter 2 is already published, and contains one study about the bioenergetics of sperm motility in the Siberian sturgeon by means of slective inhibitors of the energetic routes and by measuring the oxygen consumption rate. The study is well designed and performed, and the conclussions seems appropiate according to the results. The main conclussion is that the energy pathway more important for the sperm of this species is the phosphorilation oxidative, both in quiescent or in activated way, being important the energy accumulation in quiescent stage. This is a novel result in this species.

Chapter 3 is also published in *Biology*, and studies the bioenergetic pathways in a fish that spawns at very low temperature, the burbot. The approach also consider the changes in

mithochondrial respiration at low and high temperatures, being the first study of this type.
The study has been done by using inhibitors (for glycolisis, fatty acid oxidation, oxidative phosphorilation), and uncouplers of the oxidative phosphorilation. Also, by measuring the oxygen consumption rate. The conclussions are according to the results, and, like in the case of the Siberian sturgeon, it is concluded that most of the spermatozoon energy was derived from stored ATP that had been synthesized via oxidative phosphorilation during a quiescent but bioenergetically active state.
Chapter 4 includes a manuscript that has not yet published, and studied the bioenergetic pathways in sterlet spermatozoa, as well as the possibility of sperm reactivation, the fertilizing ability of reactivated sperm, and the possibility of improvement in the fertilization rate of reactivated sperm. The manuscript is easily readable, the methodology is sound, and the conclussions are supported by the experimental data. Indeed, it is the first report that demonstrates that sturgeon spermatozoa have the capability for reactivation after termination of the first motility period.
The general Discussion relates all the results obtained in the previous chapters, including Sperm bioenergetic facet and its relationship with motility duration, Sperm energetics in under-ice spawning fish and its thermal dependency, Phosphocreatine-creatine shuttle system supplementing OXPHOS for energy supply, and reactivation of sterlet spermatozoa and major bioenergetic pathways involved. The general discussion is well written and informative. Also, the final conclussions of the PhD are appropriate and summarize well the main results obtained. Overall, the thesis is an excellent work on fish sperm bioenergetics.

FINAL RECOMMENDATION

x☐ **PhD thesis can be recommended for defence**
☐ **PhD thesis can be recommended for defence with reservations**
☐ **PhD thesis cannot be recommended for defence**

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17th December 2021……
Date and place

Dr. Luz Pérez Igualada……
Name and signature