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: Kowalski	**Institution**: Institute of Animal Reproduction and Food Research Polish Academy of Sciences in Olsztyn Department of Gamete and Embryo Biology Poland
Name: Radosław Kajetan	
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Please describe your professional relationship to the PhD student: No relationship	**Please describe your field of expertise:** Sperm motility, spermatology, 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)**:

Sperm motility energetics is a quite large topic in terms of fish biology. Since this vertebrate group is very diverse, different strategies and energy sources are used for the maintenance of sperm movement. Energy substrates such as sugars are known to be a key for cyprinids sperm motility, whereas salmonids sperm can also utilise the lipids. This diversity results in different bioenergetics of fish sperm. These differences are visible in motility duration, triggers of the motility activation, pathway of the movements, ionic composition of seminal plasma, reproductive strategies, reproductive behaviour and many others. The PhD candidate chose two species whose reproductive biology in terms of spermatozoa energetics is relatively low recognised: Sturgeons (Siberian sturgeon and sterlet) and burbot. Using these models he studied 4 aspects of sperm motility:

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.
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.
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.

The above-mentioned studies have ambitions to fill some gap between understanding the extent of thermal compensation and strategy behind the metabolism of the fish spermatozoa. The study was carefully planned and

questions were pointed out at the beginning of the studies have significant value for better-understudying fish reproduction biology.

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 dissertation is well written. The thesis is divided into 5 chapters. The first chapter consists introduction section and at the end provide us with specific research purposes. Chapter 2 describes energy pathways associated with sustained spermatozoon motility in the Siberian sturgeon. Chapter 3 describes bioenergetic pathways in the sperm of an under-ice spawning fish, burbot emphasises the role of mitochondrial respiration in a varying thermal environment. Chapter 4 is focused on bioenergetic pathways during non-activation, post-activation, and reactivation of the sterlet spermatozoa. Chapter 5 consists of a general discussion as well as a summary in Czech and English and supplementary parts (acknowledgements, publication list and candidate info.

The dissertation is very well structured, and consist of two already published manuscripts in well-recognized journals (Journal of Fish Biology, Biology). Apart from that, chapter 4 are showing the new data ready for submission for the scientific journal.

The presented methodology, consequently repeated in three different species, allows the authors to draw meaningful conclusions.

OVERALL COMMENTARY ON THE PhD THESIS

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

The recognition of spermatozoa biology in bioenergetics are of important interest from the practical point of view. Better knowledge regarding these topics could be fruitful in terms of artificial reproduction improvements. Especially gamete handling and storing methods could be improved when more information regarding energy sources and utilisation manner will be available.

The PhD candidate aimed to assess the difference in energy supply and utilization strategy in spermatozoa of fish species that possess differences in their motility duration and spawning temperature – Siberian sturgeon and burbot. In addition to this, the possibilities of sperm reactivation or second activation in sterlet were also explored. For this, motility parameters, most important bioenergetic pathways, and reattainment of fertilizing capacity during sperm reactivation were studied.

The results of the first experiments show that OXPHOS is active during the quiescent and motile phase of Siberian sturgeon spermatozoa. Additionally, like in teleosts, the energy needed for prolongation of sturgeon sperm motility is derived majorly from pre-stored ATP that is produced via OXPHOS during the quiescent state. Moreover, this study shows no significant inputs for the glycolysis and fatty acid oxidation in the energy supply of motile and immotile sturgeon spermatozoa what was found to be important for the cyprinids and salmonids respectively.

In chapter 3 results of experiments reveal that burbot spermatozoa showed no significant effect of sperm exposure to the inhibitors of glycolysis or fatty acids oxidation on any studied motility parameter. It was also shown that at spawning temperature (+4^{o}C), burbot spermatozoal OCR could neither be enhanced by motility activation nor by uncoupler of OXPHOS at an activated or non-activated state. At +15°C, a significant reduction in OCR after exposure to NaN_3 was observed in both AM and NAM at 30 and 60 s. PhD candidates conclude that the OXPHOS in burbot spermatozoa in both AM and NAM becomes more intensive at a higher temperature.

Presented study based on the sterlet spermatozoa as well as experiments on Siberian sturgeon spermatozoa found that sturgeon spermatozoa derive energy mainly via OXPHOS as well as PCr-Cr shuttle system at quiescent state. After motility activation, the relevance of both OXPHOS and PCr-Cr shuttle system for energy supply is negligible, since the energy derived is insufficient to compensate for high ATP consumption in the flagellum.

The results of the study demonstrated that after the termination of the first round of sterlet sperm motility in AM, motility was inhibited entirely a few minutes after adding reactivation medium (RM) to the motility exhausted spermatozoon. After the incubation of immotile spermatozoa in RM, the spermatozoa were again able to be motile. However, the motility percentage, motility duration, and sperm curvilinear speed after incubation were significantly lower than in the fresh sperm. The prolonged time of incubation did not improve measured parameters of sperm motility. Despite some deprivation in the motility parameters, the reactivated spermatozoa show the ability to fertilise eggs at a similar rate as fresh sperm.

The dissertation shows 6 main conclusions:

1. A major part of the energy for motility of the spermatozoa in studied fish species (sturgeons and burbot) is derived from oxidative phosphorylation, while glycolysis and fatty acid oxidation are the least contributing pathways.

2. The oxidative phosphorylation in these fish species is actively participating in the energy supply of spermatozoa during quiescent and motile periods. However, a major part of the energy is derived from oxidative phosphorylation at the quiescent state. During motility, the energy demand of spermatozoon is too high to be satisfied by oxidative phosphorylation occurring at that time.
3. Irrespective of the sperm motility duration or temperature of the external environment, the above-mentioned energy budgeting strategy is common in the spermatozoa of studied fish species.
4. The ability to enhance an oxidative metabolism after activation of motility or exposure to an uncoupling agent is a species-specific phenomenon, which cannot be explained only by the thermal habitat history of a specific fish species.
5. After termination of the first round of motility, sturgeon spermatozoa can be reactivated. Despite the main sperm motility parameters of reactivated spermatozoa being significantly lower than ones of the fresh sperm, they possess a high fertilizing ability.
6. Mitochondrial respiration along with phosphocreatine-creatine shuttle system are the majorly contributing pathways for energy supply in sturgeon sperm at quiescent and reactivated state.
All these conclusions are supported by the results presented in the dissertation.
Overall the PhD dissertation is well written and all results fulfil the aim of the study presented in the introduction. It is my pleasure to recommend Deepali Rahi as a successful PhD candidate.

FINAL RECOMMENDATION

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

23.12.2021 Olsztyn
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Date and place

Radosław Kowalski
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Name and signature