



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

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

Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Viktoriya Dzyuba, M.Sc., Ph.D.	First name(s), surname, titles of supervisor: Jacky Cosson, Ph.D., Dr.h.c.
Title of PhD thesis: Role of regulatory proteins in fish sperm motility	

REVIEWER:

Surname: Horvath	Institution: Szent Istvan University Department of Aquaculture H-2100 Godollo, Pater K. u. 1., Hungary
Name: Akos	
Titles: M.Sc. , Ph.D.	E-mail: Horvath.Akos@mkk.szie.hu
Please describe your professional relationship to the PhD student: None	Please describe your field of expertise: Aquaculture, sperm cryopreservation

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 topic of the dissertation is obviously an original one. The study of proteins participating in the regulation of motility in fish spermatozoa has not been investigated in detail to date. Therefore, it is a pioneering work in its field with important findings that allow a better understanding of the relevant physiological processes in fish sperm motility. The studies carried out on acipenserid fish are of specific importance as these ancient species display numerous differences in their reproductive biology (including that of sperm) when compared to teleost fish. Thus, the findings described in this dissertation regarding the activation of motility in sturgeons are truly groundbreaking. Comparisons made to other fish species (carp and tench) put the work into a good perspective and allow the reader to have a meaningful comparison of the underlying physiological processes.



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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, logicity 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 essentially a collection of the articles authored by the applicant. As such, it is logically arranged, chapters (papers) follow each other in a sequential order, describing a good story of how one investigation stemmed from the previous ones. The dissertation starts with an introductory chapter including a review paper on fish sperm motility and a structured general introduction of the topics investigated in the experimental part. It is then followed by the three thematic chapters: the first describing the enzymatic activity and energy supply of spermatozoa motility, the second on sturgeon sperm maturation and the third on the role of the antioxidant system and oxidative stress in fish sperm motility. All these are essentially papers that have already been published or manuscripts accepted for publication. The dissertation is concluded by a textual discussion of the results, a summary in English and Czech and finally the various appendices (list of references, CV, etc.). As the dissertation consists of peer-reviewed publications, the originality of selected approaches and the overall quality of the work is beyond doubt.

OVERALL COMMENTARY ON THE PhD THESIS



Please write comments in extent of 1-2 pages:

This dissertation is a logical continuation of the work initiated by the group working in Vodňany on the biology of fish spermatozoa with efficient collaboration with other leading scientists of the field. For an outside viewer, a seemingly unlimited access to biological resources (e.g. sturgeon broodstocks), excellent research infrastructure and the expertise of distinguished scientists provide a perfect combination for high-quality scientific work. The present dissertation is a good example of this type of scientific activity.

Regarding the content of the work, beyond its scientific quality, it has also important implications from the point of view of aquaculture practice. In some farms working with starlet it used to be a common practice to sacrifice males and extract sperm directly from the testes to ensure fertilization. According the findings of the dissertation, this was obviously a mistake as testicular sperm needs to go through a process of maturation before it can function properly. Thus, the relevance of this work cannot be questioned.

My only criticism regarding this work is also concerning fertilization: motility and related endpoints (such as VCL or motility duration) are reported in almost all studies of this dissertation, however, fertilization tests were carried out only in one experiment (Cryobiology, 69:339-341, 2014). Fertilizing capacity is the most important characteristic of sperm, without this motility is meaningless. Thus, the reader does not have an idea how proteolytic activity or the prooxidant-antioxidant system in testicular sperm or sperm from the Wolffian ducts relate to the fertilizing capacity of sperm.

I have some specific questions regarding some of the experiments in this study:

Page 47 of the dissertation: authors postulate that urine is the dilution factor of sperm during its maturation process which is logical. Later authors state that the ionic composition of the urine and seminal fluid of sperm from the Wolffian duct is similar. They continue discussing this by stating that high K^+ and low Ca^{2+} are responsible for keeping sperm immotile in sturgeon in a dose-dependent manner. Did authors explore the threshold values of these ions? Because in this experiment, K^+ concentration in testicular sperm is significantly higher than that of either urine or SFWF. As the sperm was immotile immediately collection, therefore, the relatively low K^+ concentration is still high enough to maintain cells immotile.

Page 51-52: You state that you counted fertilization at the eyed stage. The expression „eyed stage” is common in salmonids and represents a very advanced stage of embryogenesis when the developing embryos have pigmented eyes. Are you sure that this expression can be applied to sturgeon, too? The embryo shown on Fig 1. on page 52 definitely does not have pigmented eyes.



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FINAL RECOMMENDATION

PhD Thesis can be recommended for defence

PhD Thesis can be recommended with reservations for defence

PhD Thesis can not be recommended for defence

14.06.2015, Gödöllő, Hungary
Date and place


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Ákos Horváth PhD



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Viktoriya Dzyuba, M.Sc., Ph.D.	First name(s), surname, titles of supervisor: Jacky Cosson, Ph.D., Dr.h.c.
Title of PhD thesis: Role of regulatory proteins in fish sperm motility	
REVIEWER:	
Surname: Kowalski	Institution: Institute of Animal Reproduction and Food Sciences, Polish Academy of Sciences, Olsztyn 10-747, Poland
Name: Radoslaw	
Titles: Prof.	E-mail: r.kowalski@pan.olsztyn.pl
Please describe your professional relationship to the PhD student:	Please describe your field of expertise: Fish sperm biology

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**):

Presented PhD dissertation is composed from 5 already published articles and 3 submitted to the journals. This is astonished amount of work and remarkably quality as for the PhD degree candidate. Most of presented results were already published in journals with high reputation other are submitted to such. This indicates the high quality of results and their interpretations as these manuscripts were accepted with regards to the critical evaluation in editorial boards of Journals. Almost all of presented articles might have great impact on the better technique development which may help to manage fish gametes in hatcheries. They also opened new research fields as the proteinase role in sturgeon sperm maturation. Moreover, by describing the presence of the creatine-phosphocreatine shuttle in sterlet sperm and evaluation of the relative contribution of creatine kinase and adenylate kinase in ATP regeneration in the demembrated spermatozoa of sterlet and carp during their motility period, cast new light into energy turnover in fish spermatozoa. Taking together a good understudying of basic sperm biology is prerequisite to improve fish reproduction protocols in hatcheries. This understudying let the authors to aim successful cryopreservation of sturgeon testicular sperm. This content especially reveal that author have clear vision of the usefulness of presented knowledge. Overall, the input of author into knowledge regarding sperm biology is significant.



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, logicity 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**):

First chapter goes with good introduction; a review published in *Reproductive Biology* gives good background for further work described thereafter. Review contains the recent knowledge regarding environmental signals and their translation to the sperm by second messengers. It is valuable position for those who want to get comprehensive and clear view into controlling systems of the fish sperm motility. Second chapter was lead to study of the presence of the creatine-phosphocreatine shuttle in sterlet sperm and evaluation of the relative contribution of creatine kinase (CK) and adenylate kinase (AK) in ATP regeneration in the motile phase of demembrated spermatozoa of sterlet and carp, taxonomically distant fish species in which spermatozoa differ in structure, mode of motility activation, and motility duration. This was especially interesting topic as it is known already, that fish sperm can regenerate after motility period if transferred into immobilizing conditions. These data indicate that ATP generation is present in fish sperm. Due to presented results we get to know that CK activity is dominant in both, sturgeon and common carp sperm. Moreover the CK activity is higher in sturgeon sperm which might also reflect the differences in sperm motility period present in these species (sturgeon sperm swim longer than common carp). Using the inhibitors authors shown us the possible regulatory mechanisms of motility cessation. Submitted to Plos One article reveal a great piece of work done on this physiological comparison of two distinct sperm.

Chapter 3 consist the information regarding proteolysis and their importance in sturgeon sperm maturation. This chapter have both, theoretical and practical approach both explained by well-done experiments. Chapter 4 aims to better understand the role of antioxidant shields presented in seminal plasma together with consequences of sperm expositions to osmotic shock which lead to oxidative stress. The information flow in this dissertation is satisfactory if not outstanding. Overall represent data which clearly correspond with the aims of the PhD thesis.



OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The knowledge regarding sperm motility gives us great chance to not only understand biology, but also helps the people involved in fish production. Aquaculture is recently one of the most growing food production chains. The reason of that is mainly related to two circumstances: 1 decreasing of captured fish delivery and 2 increasing the demand of healthy food. The recent „hunger“ of fish could be only covered by the developing more efficient aquaculture production traits. This could be done only when together with developments of the new production technique, fish reproduction will also gain new knowledge. The basic biology is always a servant to our human needs however we should wisely choose the reasons and tasks for their proper use. In the presented PhD we can see how to use this knowledge in the close to production area. Cryopreservation recently is demanded technique for all hatcheries which manage large amount of the priceless broodstocks fish. PhD candidate together with their co-workers published information regarding cryopreservation of testicular sperm, which was before in vitro matured. This maturation was achieved due to understanding the role of Wolffian ducts in sturgeon sperm maturation. The article from Reproductive Biology, published by the applicant together with co-workers describe briefly but essentially the effects of Wolffian ducts on sperm maturation and inhibition of maturation process by the calcium ion blocker. The publication regarding proteolytic activity although cast new light on the latter mentioned findings, bring also some hard to explain data. Nevertheless it is worth to mention that proteolytic activity of sturgeon seminal plasma was shown for the first time. The differences in the zymograms based on casein as a substrate, indicate differences in proteolytic activity. From my experience, most of these activities are related to trypsin-like proteases (casein is preferred substrate for them). Evidence of that might support the previous findings of candidate, that inhibition of trypsin-like activity might block maturation event in sturgeon sperm. It is very likely, those proteases visible in casein gels in WS extracts are related to molecules released or activated in Wolffian ducts. To classify the caseinolytic activity, simple addition of SBTI to incubation media is sufficient. Disappearing of activity in presence of SBTI will indicate the class of protease as serine-like. In this place I want to encourage the authors to future enlargement of their research to classify all enzymes present in sturgeon reproductive tract. It could be able then to identify enzymes and in the possible future, use it artificially to obtain “perfect” maturation in vitro.

Regarding the work aimed to energy turnover in sperm of sterlet and common carp I have some minor comments to manuscript. The comments were placed in revised version of manuscript and have only commentary value for further consideration. The value of this chapter is also close to practice. The knowledge regarding enzymes involved in energy production for sperm might be very useful for utility purpose. It should be possible in near future to support the energy production in fish sperm in specific “booster” buffers (which already exist but might be better in the future). It might be beneficial when preserved sperm (after chilling preservation or cryopreservation) will be used in fish reproduction. Also in cases when low sperm quality is observed such a buffers might bring the solution which allow using these samples in reproduction. The future use of knowledge related to this article might be very broad, and should be considered by the authors.




The chapter described the effect of oxidative stress on sperm shown also good practical output. The suggestion of the antioxidant use in immobilizing buffers to limit negative effect of unwanted oxydation is good direction of future experiments. Besides this practical indication also the dual role of reactive oxygen species (ROS) is very interesting finding from this part of dissertation. Understudying the role of oxygen in biology is recently the very hot topic. Antioxidants together with ROS play important role in our life. It is good to not take into account as a one is always bad (ROS) and latter only beneficial (antioxidants). Understudying the ballance existing in the basic biological mechanisms, helps to describe the fragility and outstanding organization of the life on earth.

The overall value of presented data is very high. In my opinion applicant fullfil the all criteria needed to achieve PhD title. I would like to send my warm congratulation to Viktoriya Dzyuba for the work which was presented in her PhD thesis. Please never stop looking for the true answers!

FINAL RECOMMENDATION

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

17.06.2015, Sesoko, Okinawa
Date and place

Radostaw Kowalski
Name and signature