



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: Galina Prokopchuk, M.Sc.	First name(s), surname, titles of supervisor: Jacky Cosson, Ph.D., Dr.h.c.
Title of PhD thesis: Flagellar movement of fish spermatozoa: interrelationship between physical and biochemical control	

REVIEWER:

Surname: Pacey	Institution: Academic Unit of Reproductive and Developmental Medicine, Department of Oncology and Metabolism, The University of Sheffield, Level 4, The Jessop Wing, Tree Root Walk, Sheffield, S10 2SF, UK.
Name: Allan	
Titles: Professor	E-mail: a.pacey@sheffield.ac.uk
Please describe your professional relationship to the PhD student: None	Please describe your field of expertise: 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):

This is a very nice piece of work investigating the biology of sperm activation in a number of commercially important species of freshwater fish. The candidate has done very well and has so far seen four papers published in international journals from this work. The experiments undertaken are very elegant and represent a significant amount of work. As such, I have no hesitations that this PhD submission is internationally competitive and is worthy of award. In addition to the written thesis, the candidate also provides a list of other publications and conference proceedings which demonstrate that she has been working at an international level. Because of my current background (in a medical school) I am less able to tell whether or not the results will have any application to modern aquaculture, but certainly from an understanding of the basic biology the candidate has done an excellent job.



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**):

This is a Phd thesis of some 124 pages length and therefore a perfect length for an examiner to evaluate over a couple of days. It is structured around five experimental chapters, four of which are reprints of papers which have already been published for which the student should be congratulated. The thesis has three main objectives: (1) to study the processes underlying spermatozoa maturation; (2) To investigate the coping mechanism in fish spermatozoa with osmotic and ionic activating mode, as well as in spermatozoa of euryhaline fishes, to various osmotic conditions; and (3) to describe the regulation and initiation of flagellar beating in chondrosteian spermatozoa. The objectives are wholly met in the experimental chapters presented and the results are nicely discussed in the context of the relevant scientific literature.

OVERALL COMMENTARY ON THE PhD THESIS

The thesis begins with an overview of the reproductive system of fishes and a detailed overview of the activation of sperm motility in externally fertilising species. This includes a summary of the importance of osmotic and ionic changes in the intracellular compartment of the sperm flagellum. The introduction concludes with a discussion about the physiological and structural components of the sperm axoneme which then lead to the activation of sperm motility. The introduction of the thesis then concludes with the three objectives outlined above.

The first experimental chapter of the thesis (Chapter 2) then describes the in vitro maturation of sperm from the sturgeon and highlights the importance of sperm dilution by urine and the involvement of an unidentified >10kDa substance isolated from seminal fluid. This work involved the use of video recordings to estimate the percent motility under each experimental incubation as well as the curvilinear motility.

In Chapter 3, the thesis examines the volume changes in sperm from a species which either use osmotic or ionic modes of sperm motility activation. This exploited the use of three different techniques to evaluate how the sperm volume changes under a range of osmolarities. The results compared the three techniques and found that nephelometry was the most reliable method. But the most interesting part of this work is the fact that there were species-specific difference in the way that sperm volume was altered. Again

Chapter 4 outlines the adaptations of sperm to conditions of high salinity encountered by the commercially important species tilapia which can live in environments across a range of salinities. As such, it is an excellent model organism to answer the question of how sperm

can cope with these differing environments where spawning and reproduction needs to take place. Again this involved the analysis of sperm motility parameters from digital recordings of sperm diluted into media of different salinities and osmolarities. These data showed that sperm motility was surprisingly tolerant across a range of salinities, but that external calcium ions were required to facilitate sperm activation. Also, it was noteworthy that sperm were motile over a much longer time period than that typically observed in other teleost fish. It is noteworthy that the work outlined in this chapter has been accepted for publication, although has not yet appeared in print.

In Chapter 5 the topic switches back to sturgeon sperm motility and a series of experiments are presented which seek to untangle the antagonism between potassium ions and osmolarity. This uncovers a previously unexplored signalling pathway in sperm motility activation whereby the potassium inhibition of sperm motility can be by-passed if the sperm are first exposed to a high osmolarity shock.

In the final experimental Chapter 6, the candidate uses high-speed video micrography and image analysis of sperm flagella to investigate the initiation of motility in the sperm of the Sturgeon. This shows that the time period needed to achieve full wave propagation ranges from 0.4 to 1.2 seconds. Moreover, the sequence of events typically seen is described in great detail. This paper has already been published and provides a good platform upon which further studies could be established.

The thesis is concluded with Chapter 7, which includes a general discussion which nicely pulls together the data from the five experimental chapters. In some ways it is disappointing that the candidate not present a section which outlined avenues for future work. But as it stands the thesis is a very solid piece of work and certainly worthy of a PhD.

There are some minor typographical issues to correct, particularly in the Table of Contents where the page numbers of either missing or incorrect. These should be corrected.

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

10-06-2016 SHEFFIELD
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Date and place

A. A. Farley
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Name and signature
PROFESSOR ALLAN FARLEY



Confidential

Review of USB FFPW PhD Thesis

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Title of PhD thesis: Flagellar movement of fish spermatozoa: interrelationship between physical and biochemical control	

REVIEWER:

Surname: Lindemann	Institution: Oakland University Rochester MI 48309 USA
Name: Charles B.	
Titles: Professor	E-mail: lindeman@oakland.edu
Please describe your professional relationship to the PhD student: Never met	Please describe your field of expertise: Biological sciences

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**):

This doctoral thesis consists in large part of four published reports and one prepublication accepted manuscript. In addition, an introduction to the work precedes the published work, and a general discussion, acknowledgements and CV follows the published work.

The introduction is good, it is fairly well written and understandable, with only 2-3 minor grammatical errors per page. The introduction is fairly comprehensive in providing a good survey of the current state of the field and provides an extensive survey of the relevant literature. Similarly, the discussion is fairly clear and understandable and does a good job of conveying the major findings of the five scientific reports that constitute the body of the thesis. The discussion could benefit somewhat from inclusion of a figure or figures that summarize the findings in a more visual context, or alternately a summary table that shows the common elements and differences between the activation schemes in the different fish species that have been studied thus far.



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**):

All of the research included in the thesis has been published or accepted for publication. This is normally a very good thing, as it means the work has passed review and been accepted by experts in the field. There are two major problems with this thesis, both related to the main body of research. The first problem is that all of the papers are multiple authored, and the doctoral candidate is primary author on only two of the five papers. This raises a serious question as to the actual contribution of the doctoral candidate to the main body of work. Without a clearer delineation of the role of the candidate in each of the reports it is not possible to evaluate whether, or not, the candidate has made significant conceptual contributions to the field. This presents a big problem in formulating a fair evaluation of this thesis, as making a substantial contribution to the field is the general standard of evaluation for doctoral level work.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

I would strongly suggest to the candidate's committee that this question must be extensively addressed in the thesis report for each of the published works. I would also suggest that it is not sufficient for a doctoral candidate to simply have participated in a technical capacity in a piece of work to claim it as part of a thesis, there must be an intellectual component that includes the design and planning of the experiments, as well as substantial participation in the writing and presentation of the work. This issue is definitely in question in the present case, and must be resolved. If the answer is that the candidate provided some technical support, such as carrying out the filming or composing a figure, then the paper should not even be a part of the thesis, as it is predominantly the project and intellectual contribution of a different principal author.

The second major problem is this. Unless otherwise stated, the order of authorship normally reflects the order of intellectual contribution to a published report. Therefore, I must make the assumption that the two papers on which the candidate is the first author are, for the most part, the actual basis of the thesis research. On that basis, I would conclude that the candidate's research accomplishments thus far appear to be very preliminary, and in this reviewer's view, insufficient to merit granting a doctoral degree.

Both of the first-authored papers are largely descriptive. They utilize techniques that have been in general use for at least 40 years. The only novel technique utilized was the high speed filming set up used in the last report. It is novel only in the sense that it uses new camera technology that was not available previously. High speed filming is not in itself anything new. Although the regulatory pathway in sturgeon has not been delineated, there is a wealth of sophisticated work that has been done on activation of sperm motility in other organisms, both vertebrate and invertebrate.

The techniques used to investigate the activation pathways in sea urchin and mammals are all available in published studies. The statement made in the introduction of the final chapter that "in



contrast to mammalian sperm" fish sperm are inactive in the male reproductive tract, is incorrect (second paragraph page 91). Mammalian sperm are also quiescent in the testes and epididymis and also require activation, which normally occurs upon ejaculation. I have read many excellent papers on the activation process in mammalian sperm and have firsthand experience with activating rat and mouse sperm. The same techniques and methodologies that were used to sort out the activation process in sea urchins and mammals are available to be used to sort out the activation scheme in sturgeons. The results presented in this thesis, while interesting and relevant, are just a start based on a very limited number of experimental approaches. I do not see the depth of investigation of the problem that I would expect of a doctoral thesis. Furthermore, the lack of modern techniques will be a handicap to the candidate when it comes time to apply for positions.

There are many questions that could be answered by a more extensive investigation of the activation scheme. Does it work through a known signaling pathway, either calcium mediated or cAMP mediated? Does the acquisition of motility involve phosphorylation of a constituent motility protein? Are there voltage or bicarbonate gated channels in the membrane as are found in mammals? Nature is usually at least somewhat conservative in the evolutionary development of control pathways. Is there anything expressed in sturgeon gametes genetically related to the Catsper channels of mammals? The study so far only points to the first step in the activation sequence, and leaves so many obvious questions unanswered. Why do fish sperm lose motility so quickly? Is it the axoneme that stops working, or does the membrane leak ATP? This could easily be answered with some follow up experiments that are technically within reach of the candidate.

I have supervised the research projects of undergraduate students for 40 years and have served on five doctoral committees. The two studies that compose the first-authored work of this candidate are roughly at the same level as accomplished by several of my best undergraduate research students. Both papers are good solid studies and they do make useful contributions. Those two studies, considered by themselves, do not come up to the level that I expect, and have encountered, in all five of the successful doctoral dissertations that I have evaluated. I cannot fairly evaluate the extent that the work in the other three studies should also be considered as the intellectual property of this candidate. As such, I cannot render a valid opinion on whether or not the additional papers elevate the thesis to a sufficiently high level of academic achievement to warrant granting a doctoral degree. The members of the candidate's thesis committee and the candidate's mentor are in a much more informed position to evaluate the extent of the candidate's contributions to the other three papers. I trust that the members of the thesis committee will be able to render a fair judgement on that issue.

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

Dr. CHARLES B. LINDEMANN

15.6.2016, Rochester, MI, USA

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Date and place

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Name and signature