



**Confidential**

### **Review of USB FFPW PhD Thesis**

<b>First name(s), surname, titles of the PhD student:</b> Hadiseh Dadras Asyabar, M.Sc.	<b>First name(s), surname, titles of supervisor:</b> Assoc. Prof. M.Sc. Borys Dzyuba, Ph.D.
<b>Title of PhD thesis:</b> Temperature dependency of sperm motility in different fish species	
<b>REVIEWER:</b>	
<b>Surname:</b> Labbé	<b>Institution:</b> INRA, Fish Physiology and Genomics Bat 16A Campus de Beaulieu F-35000 Rennes
<b>Name:</b> Catherine	<b>E-mail:</b> <a href="mailto:catherine.labbe@rennes.inra.fr">catherine.labbe@rennes.inra.fr</a>
<b>Titles:</b> Dr.	
<b>Professional relationship to the PhD student:</b> Supervision of 2 internship of Hadiseh in my group.	<b>Please describe your field of expertise:</b> Gamete biology, fish physiology, cryobiology, embryo development

### **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 PhD study is devoted to the effect of temperature on sperm motility in fish. Its purpose is to better understand the behavior of spermatozoa upon activation at sub-optimal and optimal temperatures, and to identify the biochemical actors that may be modified during spermatozoa movement. The goal of the thesis as it is written P.24 is to “address the current lack of knowledge” in the field of temperature and sperm motility in fish. It provides a nice set of data in four fresh water species of high economical importance in aquaculture that are rainbow trout, sterlet, common carp, and Eurasian burbot. It is interesting that chapter 2 compares species bearing different reproductive conditions, and that their spermatozoa have different motility duration. There is a logical thread consisting in assessing the peroxidation after motility completion. The chapter 3 dedicated to changes in lipid composition after sperm movement provides interesting data on a metabolism that is rarely studied in fish, and which deserves further work thanks to the interesting results obtained in the present work. The last chapter is devoted to a hard winter spawning species, and the study provides a better knowledge on the control of motility activation in this species, although the applied impact is pending. The whole impact of the thesis is that it opens several scientific fields for further studies on sperm metabolism in different fish species,



thanks to well raised questions and well performed experiments. The whole work was developed in a field where very few data were available. The data that were produced in this thesis are original. They provide both basic knowledge and information that can be further developed for application in aquaculture.

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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 thesis manuscript of Hadiseh Dadras is well written, well presented, and the objectives are clearly stated. I find especially interesting that Hadiseh wrote a review article on her PhD topic. This should be taken as an example. This gave her a strong expertise and scientific background in her field, and this likely gave her more authority in the writing of her experimental work. This is clearly emphasized in the introduction that Hadiseh wrote at the beginning of her thesis manuscript. It is especially interesting that Hadiseh took the studied parameters one by one and explained the purpose of her focus on these parameters. First Hadiseh explained well that the effect of temperature on sperm motility variable is highly variable because of differences in the fertilization mode of the studied fish species: motility duration is different, spawning temperatures are different. Second, she pointed out that comprehensive investigation of the lipid peroxidation in relation to temperature is of interest with regards to the above mentioned particularisms. Third, she explained well that lipid peroxidation led to raising the question of lipid metabolism and thereby to lipid composition after motility. Last she developed the specificity of burbot spermatozoa whose motility can be activated in seminal plasma with the sole change in temperature. The order of the chapters which followed this nice introduction is logical. I will comment in the next section about the main achievements obtained in these experimental chapters. Two experimental articles on carp are published, whereas two other articles are written, and in my opinion almost ready to be submitted. The quality of the final general discussion is at the level of the introduction: well written, taking all parameters one by one and discussing the main information brought by the work. The overall level of the manuscript is good and it does not require any changes for the defense. Before the last articles are submitted, they may require slight English editing, but the whole thesis manuscript is absolutely understandable as it is.

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### **OVERALL COMMENTARY ON THE PhD THESIS**

**Please write comments in extent of 1-2 pages:**

This thesis work by Hadiseh Dadras produced one review in *Aquaculture Research* in 2017, one original paper in *Journal of Thermal Biology* in 2017, and another one in *Animal Reproduction Science* in 2016. Two other articles are in a state of almost ready for publication. Hadiseh is first author of all these publications. She has also co-authored 9 other articles including one as first author, three of them having a large audience before the international fish community.

Hadiseh has presented her thesis work as poster at three international conferences gathering the fish community (*Aquaculture Europe*, and *Biology of Fish Gametes*).

Hadiseh has performed 2 internships in a foreign laboratory, at INRA (France) (2016 and 2017). She has participated to one training school in Faro (2015) on reproductive biology in fish, and has led one summer school projects at her university (2016).

These accomplishments sign the excellent quality of Hadiseh's work during her PhD, and illustrate her dedication to her work.

I have several points for discussion that I want to raise here. I want to clearly state however that my comments in this section are intended for general scientific discussion, and questions that may be addressed during the oral defense. None of my comments deserves that the present manuscript be changed.

- For the oral defense, it might be interesting that a general table (or picture) be built, with the 4 studied species presented according to their spawning temperature, motility duration, and knowledge about sperm metabolism. This may provides a nice introduction to the study, and provides readily rationale on the choice of the species (but this is up to Hadiseh and her supervisor, this is not mandatory).

**- Lipid phase transition:**

In her review chapter, Hadiseh comments about the lipid phase transition at low temperature. It should be pointed out that this is a specificity of mammalian spermatozoa. Fish membranes are much more fluid, and no phase transition was reported above 0°C in trout for example (see Labbé et al, *Cryobiology*, 1997). In her interspecies comparison, Emma Drobnis (1993) showed that Shrimp spermatozoa do not have a straight phase transition above 0°C either. Beware that poikilothermic organisms are adapted to temperature changes. One clear illustration is that mammalian spermatozoa have to be handled in cryoprotectant before they can be stored at 4°C, whereas almost all fish spermatozoa can be stored at 4°C without cryoprotectant.

**- Antioxydant activity in fish spermatozoa**

The discussion at the end of p. 46 is very interesting. Do you think some species may have more natural antioxydants than others, depending on their diet? As a hint, when you extract lipids from trout spermatozoa, the lipid fraction is pink with all the carotenoids stored in the membranes. Is it



the same in sterlet?

**- Lipid metabolism in fish spermatozoa:**

In her general introduction and in the discussion of her PhD work (p. 57), Hadiseh states about the example of sea urchin spermatozoa which are using lipid globules as energy sources for motility. I want to emphasize that we cannot draw a straight line between this work and carp lipid metabolism during motility. Indeed, the motility duration is highly different between sea urchin and carp. Lipid metabolism does not have time to produce energy in the case of carp. It is likely that the observed changes in lipid proportions in carp are due to lipid peroxidation, as demonstrated in the first experimental paper of Hadiseh. Besides, carp spermatozoa do not have lipid globules. For example, how would you assess the fate of the "metabolized" lipids during motility? Why would there be any need for fluidity changes? The interesting items raised in the discussion of the article may be further developed during the thesis. Another comment about this issue: how do you explain the quite high variability in phospholipid composition in immobile spermatozoa? One difficulty of this kind of study is that everything has to be expressed as a % of the total components. Then, everything is about proportions, not about absolute contents. But this is cautiously addressed in the article.

**Spermatozoa activation with temperature in burbot**

If possible, this paper in preparation will need an additional set of data corresponding to the response curve of motility activation with temperature. The whole paper cannot hold if this curve is not shown (stating the observation in the method section is not enough). These data will allow to justify why the whole article is based on 4 and 30°C. Also, the presentation table 2 should be modified so that the ions involved are displayed, and not the A, B, C, D, E name of the media. In all, this table should be better explained in the legend, because it took me some time to understand its purpose. I figured out that minimum EGTA is to PREVENT motility activation, whereas minimum Ca<sup>2+</sup> is to ACTIVATE motility. The experiment which was made to obtain this information is not well described. In figure 2, the velocities cannot be expressed as %. Some results show up in the discussion (EGTA, p. 77) and I could not find them in the results.

**Miscellaneous:**

- It is unfortunate that the time after activation at which spermatozoa were used for enzymatic activity in chapter 2 is not stated. One can guess from the lipid article that it was after cessation of movement. This information may be added for publication of the trout and sterlet data.
- In the sterlet paper (p. 39), Sterlet spermatozoa are kept immobile in a sperm motility inhibiting medium, and in a non-activation medium. Which one was used? Besides, it may be good to indicate that the time in the immobilizing medium prior to biochemical studies is the same as the activated samples (if so).
- In the sterlet paper (p. 44), I would advise to use the same scale for enzymatic activity in the 2 species. This would emphasize the species differences. Same for TBARS.

As a conclusion, this thesis by Hadiseh Dadras is well written, and it contains a huge experimental work that is well presented and well discussed. Hadiseh proved in this work that she is able to



defend her work at international meeting, to write articles, and to supervise summer school projects. She is an early stage researcher who fully deserves the right to defend her PhD. I highly recommend her for PhD defending.

### ***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

Date and place

...19 Juillet 2018 Rennes.....

Name and signature

Catherine Labbé



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**Review of USB FFW PhD Thesis**

<b>First name(s), surname, titles of the PhD student:</b> Hadiseh Dadras Asyabar, M.Sc.	<b>First name(s), surname, titles of supervisor:</b> Assoc. Prof. M.Sc. Borys Dzyuba, Ph.D.
<b>Title of PhD thesis:</b> Temperature dependency of sperm motility in different fish species	
<b>REVIEWER:</b>	
<b>Surname:</b> Kowalski	<b>Institution:</b> Institute of Animal Reproduction and Food Research Polish Academy of Sciences in Olsztyn
<b>Name:</b> Radoslaw	
<b>Titles:</b> PhD	<b>E-mail:</b> <a href="mailto:r.kowalski@pan.olsztyn.pl">r.kowalski@pan.olsztyn.pl</a>
<b>Please describe your professional relationship to the PhD student:</b>	<b>Please describe your field of expertise:</b> 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):

The temperature is the main factor affecting the physiology of all organisms. During in vitro fertilization of the fish eggs, there is a great importance of maintaining a stable temperature, related to the biology of the species. Moreover, manipulation of the temperature during eggs incubation lead to the changes in their development level. Therefore in hatchery practice, we use the specific value of the day-degree to measure approximately time needed for each step of the development of the egg. The effect of temperature is related to several actions among which the enzymatic activity and ROS production are one of the most crucial for physiological events. Since temperature shown their importance during embryo development, it should also have an important role in gamete fertilization. However, to date, the only limited study focuses on the effect of temperature during this very short, but crucial period of fish reproduction. Therefore the observation of sperm physiology in different temperature has importance in both, basic biology field as well as in the hatcheries practice. The Ph.D. thesis focused on the sperm motility parameters including the percentage of motile sperm, duration of motility and swimming velocity as the main prerequisites of quality and fertilizing ability of fish sperm in different temperature regimes. As the model fish, the commercially important species was chosen such as common carp and sturgeon (sterlet), rainbow trout. Moreover, one chapter was devoted to the new species in



aquaculture of high valued meat, the burbot. Choosing such important species from the practical view as well as the well-recognized model species (common carp and rainbow trout) makes efforts of the author greatly appreciated by both, practical and theoretical points of view. Overall I judge the presented PhD thesis as valuable input into the fisheries sciences from the field of fish reproduction.

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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 structure of thesis is clear and logically formed. The first chapter consist published review which is always beneficial experience prior to detailed work in the field. Knowing recent knowledge helps to cover the unknown area. In this view, the introduction to the topic is very appropriate and helps to understand the purpose of the following experiments. At the end of the first introducing chapter we can find the 3 goals of presented thesis:

1. To investigate the effect of different in vitro temperatures on motility parameters and antioxidant enzyme activities in common carp *Cyprinus carpio*, rainbow trout *Oncorhynchus mykiss* and sterlet *Acipenser ruthenus* spermatozoa.
2. To study the effect of temperature on the lipid composition of activated spermatozoa in common carp *Cyprinus carpio*.
3. To explore the pattern of sperm motility activation in burbot *Lota lota* in relation to temperature.

The goals were achieved in the 3 already published works in prestigious journals such as *Aquaculture Research*, *Journal of Thermal Biology*, *Animal Reproduction Sciences* and two unpublished manuscripts. All the results described in the thesis very well correspond with desired aim of the study and significantly enlarge our understudying of the temperature effect on the fish sperm motility.

The first goal was presented in chapter 2 and consist the data regarding the effect of different activation temperature on antioxidant enzyme activities and motility of common carp, rainbow trout, and sterlet sperm. The presented data are comprehensive and provide good insight into the fish sperm motility at different temperature regimes. The 3rd chapter was aimed to better explain the common carp sperm behavior at different temperatures. The study for the first time shows differences in common carp sperm fatty acid profile changes related to the temperature. Linking these data with motility records allowing the author to better explain changes in motility pattern at different temperatures. Chapter 4 describe in details motility pattern of the burbot sperm in different media at variable temperatures. This work shows for the first time that activation of burbot sperm might be triggered only by the temperature. The molecular mechanism of such activation should be urgently investigated as it is knowledge of high practical importance. Overall, in my opinion, the Ph.D. thesis presented by Hadiseh Dadras Asyabar fulfills the objectives of the work, as well as meet the standards of academic excellence.



### **OVERALL COMMENTARY ON THE PhD THESIS**

**Please write comments in extent of 1-2 pages:**

The temperature effect on animal reproduction is an important topic, especially in the global warming realities. Better understudying of the temperature actions on the gametes helps us to prepare conservations activities corresponding to the prospected effects of the earth climate. Recently many researchers have focused on the ocean rising temperatures and their effects on the aquatic species, especially corals. However, also inland waters will be on the pressure of elevated temperatures. In this view, the presented thesis presents valuable input into the global science at the time of global changes. This work has also importance more closely related to the practice. The temperature of water used during fertilization might trigger „good“ or „bad“ performance of gametes, therefore such a knowledge is of high priority for all hatcheries managers.

Presented results clearly show that action of temperature is not only a simple elevation of metabolic rate. The specific organism has adapted to the specific temperatures, which we are able to observe on the model comparison between common carp and rainbow trout. This work shows that rainbow trout sperm velocity which is the main quality indicator of fish spermatozoa is highest at 14°C whereas common carp sperm, as well as sterlet sperm, swims fastest at 24°C. However, at this point, it is hard for me to understand the summary part written at page 74: „In the current study on carp, the highest motility percentage at 4 and 14° C, and the highest swimming velocity at 14° C was observed at 30 s post-activation.“ I cannot see any differences in initial sperm motility at all temperature (Journal of Thermal Biology), moreover, highest initial (statistically important) we observe at temperature 24°C. This point needs to be clarified by the author. The other minor comments on the thesis are related to the providing new explanation of the observed data. Especially interesting for me was:

Page 55, decreasing of ALA (18:3, n3) in 24°C might be also related to the strong antioxidant action of ALA. It is possible that in higher temperature decreasing of ALA content is related to the free radicals neutralization. What should be pointed out at this place is, that ALA is both, lipid and water-soluble (so-called universal antioxidant) and has 400 times the antioxidant strength of vitamin E and C combined. On the other hand decrease in DHA (22:6, n3) could be explained by the lowest stability level of this, the longest, unsaturated fatty acid towards elevated temperature and free radicals occurrence.

Page 46 sentence: „One explanation probably could be the fact that presence discrepancy in antioxidant capacity of sperm in different fish species.“ Despite this conclusion is correct the other explanation could be also a difference in the fatty acid content of sperm membrane. This is highly visible in TBARS between rainbow trout and sterlet. Rainbow trout sperm has a high content of polyunsaturated fatty acids (including DHA) which easily become oxidated. Sterlet sperm is probably less abundant in EPA and DHA.

Another comment I would like to include in this revision is related to a presentation of the data in a prepared manuscript from chapter 4th. My advice is to always present the data in self-describing manner. Therefore, on page 66 and 67, the figures should be rearranged to shows the CASA parameters changes in different activation media. This makes easier to discuss the effects of media on motility pattern/CASA parameters. In the present form, it is impossible to compare statistically the data obtained in different activation media. The data should be presented in clearly arranged





order, so readers will be able to see the main results of burbot sperm motility, which are:

1. Effect of EGTA addition on the suppression of the high temperature derived sperm activation.
2. Effect of Ca addition to isotonic media on sperm motility.
3. Minimum Ca concentration necessity to activation in low and high temperature.

Concentration on these results, quite well explained in summary at chapter 5th, helps to improve the first outlook of the prepared manuscript, which is always a key factor in the successful revision process. Moreover, use of WOB (wobbling) parameter in this study needs to be better explained/elaborated. This CASA parameter is not commonly used in sperm motility pattern description.

Judging the candidate activities on the field of science it is also worth to mention, that Hadiseh Dadras Asyabar participated in numerous scientific projects and published 8 manuscripts listed by the Scopus database. According to Scopus database, his Hirsh index is equal 3 which is very good results as for young researcher.

Despite all of my criticisms towards presented Ph.D. thesis, the overall scientific value of presented results is high. It is my pleasure to recommend Hadiseh Dadras Asyabar as a successful Ph.D. candidate.

### **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

29.06.2018, Olsztyn, Poland  
Date and place

  
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Name and signature