



**Confidential**

**Review of USB FFPW PhD Thesis**

<b>First name(s), surname, titles of the PhD student:</b> Vitaliy Kholodnyy, MSc	<b>First name(s), surname, titles of supervisor:</b> MSc. Sergii Boryshpolets, Ph.D.
<b>Title of PhD thesis:</b> <i>Sperm/egg interaction in freshwater fish: influence of environment on fertilization process</i>	
<b>REVIEWER:</b>	
<b>Surname:</b> Asturiano	<b>Institution:</b> Universitat Politècnica de València (Valencia, Spain)
<b>Name:</b> Juan F.	
<b>Titles:</b> Prof. Dr.	<b>E-mail:</b> jfastu@dca.upv.es
<b>Please describe your professional relationship to the PhD student:</b> No one until now	<b>Please describe your field of expertise:</b> Reproductive physiology of fish. Spermatology and fish sperm physiology. Sperm cryopreservation.

**QUESTIONNAIRE**

***Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research***

The research reflected in this PhD Thesis has a logic basis and well planned experimental design. It has been carried out with three very different fish species, covering a wide evolutionary range into the fish group. The level and originality of the works produced is very good and fully competitive in the international context of this area of research. First clear evidence is the fact of the publication of the introductory part as a review paper in the journal *Reviews in Aquaculture*, which has the highest impact factor in its category.

Moreover, the results obtained in the following chapters are in process or will be published soon. Some of the conclusions reached in these works have been often considered as potential facts in fish spermatology, but nobody tested the existence of many of these basic effects of female gametes on the spermatozoa motility except in a very few fish species.

The different species used here, and the different results obtained with them, makes evident the species-specificity of these activation-navigation mechanisms, which have importance from an evolutionary point of view.

The conclusions reached in this work will serve as a solid and promising base for future studies.



---

### ***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**):

In general, the structure of the manuscript is good and clear. General introduction has been basically substituted with a previously published review paper, which seems an excellent way to introduce the main subject of the work.

The following chapters (2-4) concentrate the experimental research and its results. With logical differences, all these works (chapters) have a similar experimental design, trying to evaluate the effects of several factors related with female gametes, female fluid or environmental conditions (i.e.: osmolality, ions concentration) on the motility of the spermatozoa, considering as well the effects on the navigation and attraction effects over the sperm cells.

The structure of all of them is clear and comprehensive, and both texts and graphic materials are of high scientific quality.

The objectives of the different chapters cover logically the main objectives of the thesis. Logically, the obtained results open new questions and suggest new experimentation that should be faced in the future.

---

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

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

First of the chapters is a brief introduction to the main subject of the Thesis, followed by a review paper published in *Reviews in Aquaculture*, giving a quite complete view of the factors involved in the sperm activation of freshwater fish species. Many of them are experimentally faced in the following chapters.

In the second chapter the candidate carried out several sets of experiments to evaluate the effects of ovarian fluid and calcium concentration in the rainbow trout sperm motility. They probed that the effect of ovarian fluid on the sperm activation can be explained by chemokinetic effects caused by changes on the osmolality and calcium concentration. They found as well that the ovarian fluid modulates the path linearity of the spermatozoa, and has a positive effect on their fertilizing



ability, improving the basal non optimal motility of the sperm cells and causing attraction and trapping of spermatozoa, changing the direction of their motion. These conclusions have been often considered in fish spermatology as potential but probed in a very few fish species. The conclusions reached in this work will serve as a solid and promising base for future studies.

The third chapter follows the same line of research, in this case using sturgeons as experimental model. Motility and chemotaxis assays, as well as an in vitro fertilization trial were carried out. Results were different to found with trout sperm, suggesting the existence of taxa-specific egg-sperm interaction mechanisms. Lower concentrations of the ovarian fluid made longer the sturgeon spermatozoa motility but did not affect the spermatozoa trajectories. However, fertilization trial evidenced that the presence of ovarian fluid prevented the eggs from losing the fertilizing ability due to the contact with water by preventing its activation, as well as promoted the spermatozoa to fertilize the eggs during longer period of time. These are innovative results on the fish egg-sperm, giving a more evident role to the female factors.

The fourth chapter is dedicated to similar experiments with common carp gametes. In this species, ovarian fluid reduced the curvilinear velocity of spermatozoa and altered their motility linearity pattern, while attraction effect was dependent of external calcium. Again, these results are evidencing the specificity of these mechanisms depending on the fish species, probably reflecting an evolutionary process.

A few mistakes/suggestions have been found through the manuscript:

Page 9 the only candidate

Page 9 + 10 + 41 + 72+ 73 freshwater

Page 49 Check subsection title (missing word)

Page 51 patterns / up to

Page 56 with characteristic velocity of spermatozoa (repeated)

Page 57 peculiar / characteristic?

Page 57 no (repeated)

Page 58 positive taxis and trapping were observed

Page 99 h, not hrs

Page 101 s, not sec

## **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**



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

Valencia, July 22<sup>nd</sup>, 2020

Prof. Dr. Juan F. Asturiano