



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Yu Cheng, M.Sc.	First name(s), surname, titles of supervisor: Prof. Dipl.-Ing. Otomar Linhart, DSc.
Title of PhD thesis: Sperm quality affects the fertilization in freshwater fishes	

REVIEWER:

Surname: Butts	Institution: School of Fisheries, Aquaculture and Applied Sciences, Auburn University, USA
Name: Ian AE	
Titles: Asst. Prof. (Assoc. Prof starting 1 Oct)	E-mail: iab0007@auburn.edu
Please describe your professional relationship to the PhD student: None.	Please describe your field of expertise: I work on very similar studies but with different fish species.

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**):

Wild-caught fish stocks are declining, and as the world population grows, the demand for high-quality protein is increasing. New research shows that fish, crustaceans, mollusks, and other aquatic animals account for ~20% of total animal protein consumed by the global population. Aquaculture is therefore playing a pivotal role in global food security, the environment, and human health. Because the aquaculture sector is rapidly growing and is relatively new compared to the livestock sector, it offers tremendous opportunities for technological innovation to further increase sustainability to meet protein demands. Thus, this PhD project by Yu Cheng is helping to address this area. More specifically, the PhD thesis is titled “**Sperm quality affects the fertilization in freshwater fishes**” and focuses on breeding techniques and advanced sperm performance guidelines to improve hatchery production efficiencies and outputs. Overall, the research is scanning several different areas of reproduction and hatchery science. In research **Chapter 2** different embryo incubation techniques were tested to improve future research outputs, especially by increasing replications, while **Chapter 3** worked on deciphering how sperm density, egg density and water density impact embryo outputs. Research **Chapter 4** was extremely comprehensive (4 major study objectives), with the overarching goal focusing on sperm management techniques for the model species, zebrafish. Research **Chapter 4** again focuses on sperm management, testing different storage times in an immobilizing solution and swapped fluids between good vs. bad quality males to improve cellular quality.



Chapter 5 tested different extenders and activation solutions. Finally, research **Chapter 6**, was highly novel (and my favorite chapter of thesis), where sperm methylation, via sperm aging, was linked to offspring performance. **Chapter 1 and 7** were extremely thorough reviews on “**Molecular and cellular attributed of fish spermatozoa and approaches to improve the quality of cells**“. For dependant variables, Yu Cheng measured sperm performance using computer assisted sperm analyses system (CASA), seminal plasma parameters and did a lot of assisted reproduction. This was also linked with new and expanding sperm epigenetic research. Together, this applied/basic science is highly novel on a global scale and shall improve our ability to freeze/thaw cells, and fertilize eggs, and assess reproduction for various aquatic species.

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 PhD thesis by Yu Cheng consisted of seven chapters, two of which were overviewing the literature and another series of studies were done of experimental nature that resulted in 7 publications – wow! Here, I quickly overview the individual chapters. **Chapter 1**. Is a general introduction to the thesis in terms of sturgeon reproduction, sperm/hatchery management and damages to sperm and associated biomarkers. Here, the goals and major aims of the research have been highlighted, and overall, it was a very nice job (it has been forwarded to my lab at Auburn University to read, as the section on Epigenetics was very nice). **Chapter 2** was a nicely performed applied hatchery study published in *Animal Reproduction Science* (2015) aiming to compare incubation methods in static petri dishes vs. more commonly used systems. I feel like this study provided a nice starting point for the thesis, as we really need controlled procedures that enable more replication to conduct studies on fertilization dynamics, etc., **Chapter 3**, published in *Aquaculture Reports* (2020), was a perfect contribution to the literature as we really need to do a better job standardizing sperm during fertility. Thus, this chapter really elaborates on the importance of not only sperm to egg ratios, but also egg density, and sperm to water volumes. Together, this will make research more repeatable. Research **Chapter 4** then consisted of a series of studies on various model and aquaculture species. The research generated from these chapters was published in three high-quality and respectable journals, including, *Animals*, *Aquaculture* and *Theriogenology*. In this set of papers, I really enjoyed the sequential approach, especially for the zebrafish work, where 1 study lead to the next through a series of “small-scale” studies. Together, these data this will help the biomedical community standardize their hatchery methods more precisely. The paper on sterlet that swaps seminal plasma, showing bad quality sperm can be improved with good quality plasma, etc., was highly innovative, and has implications for studies on cryptic female choice and associated sexual selection processes. Next, **Chapter 5**, is a traditional sperm study that tests different extenders and activation solutions. Results can be transferred directly to industry for improving common carp production. Finally, **Chapter 6** is in my opinion the best quality paper of the thesis (published in *International Journal of Molecular Sciences*) as it combines new advances in molecular biology (methylation) with commonly used



hatchery techniques, clearly showing that sperm aging is a problem that can impact future offspring performance. Finally, **Chapter 7**, gives a general conclusion, and although, a nicely written section I would have enjoyed a more of a “*what would be the next logical research steps*” to solidify overall comprehension. Nevertheless, based on the above, this thesis seems to address aspects of sperm biology at a high-level of comprehension. I enjoyed it immensely and I wish this very keen student successes in the future.

OVERALL COMMENTARY ON THE PhD THESIS

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

Chapter 1:

This is a very comprehensive review. My only concern is with the flow. It sort of bounces around, from topic to topic. I believe this could be improved by following the topics in the order of the thesis or was this the initial intent. Secondly, I believe it would be nice to have a section with each model system and why it was chosen for the research.

Chapter 2:

I would have suggest individual females being reared separately, so I am glad it was done using this approach.

My only complaint about this chapter is I would have liked to have seen better pictures of the systems and maybe from different angles. For instance, the trough system looks very nice, but it is hard to understand how it was constructed. Thus, making the word not as repeatable.

Chapter 3: This was a very nice chapter, and something I have also envisioned doing for various fish species. For improvements you could have added a table summarizing sperm to egg ratios in different fish and adding the sperm/mL and egg per trials, etc., I did something like that In Butts et al., 2012, but, that was 10 years ago. Surely many studies have been done to update.

Chapter 4:

Zebrafish: Very comprehensive study. I have no issues and enjoyed reading it. For improvement, since there are so many small studies, it would have been nice to have a table or schematic summarizing the study design and treatments for each trial. I am glad you incorporated the flagella beating.

Catfish: This was a very nice study, I have nothing to add.



Sterlet: Again, a wonderful piece of literature. I believe this study will springboard many studies on sexual selection as well and hatchery techniques.

Chapter 5: Another great study. Your figures are very pretty in this chapter, but it is a pretty they all did not contain color.

Chapter 6: This was my favorite chapter of your thesis, as I really see this area of science evolving. Your figure 1,2, and 3 are very beautiful.

Chapter 7: Your general summary would have benefited with a “what’s next” for each of the research chapters. Besides that, is was a nice summary.

FINAL RECOMMENDATION

- X ☐ PhD thesis can be recommended for defence
☐ PhD thesis can be recommended for defence with reservations
☐ PhD thesis cannot be recommended for defence

14 July 2022

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Date and place

Ian Butts

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Name and signature