



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Jindřiška Knowles, Ing.	First name(s), surname, titles of supervisor: Peter Podhorec, Mgr. Ph.D.
Title of PhD thesis: Using of prolonged releasing drug systems in fish reproduction	

REVIEWER:

Surname: Zarski	Institution: Institute of Animal Reproduction and Food Research of Polish Academy of Science, Olsztyn, Poland
Name: Daniel	
Titles: Dr.	E-mail: d.zarski@pan.olsztyn.pl
Please describe your professional relationship to the PhD student:	Please describe your field of expertise:

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**):

In her thesis, Jindřiška Knowles attempted to verify the effectiveness of sustained hormone release technology (mGnRHa emulgated/encapsulated using poly [lactic-co-glycolic acid]; abbreviated to: PLGA). Technology of sustained hormone release, especially gonadolibersins which are having a short half-life in the blood stream of fishes (usually less than 24 h) has already been tested in many marine species. However, there is little known about application of sustained release of the hormonal preparations in reproduction of freshwater fishes, what makes the cycle of the publications original contribution. In this thesis several research strategies has been incorporated with various analytical techniques what makes the studies up-to-date scientific ventures. From among all the data presented the ones related to the application of the mGnRHa-PLGA in cyprinids where PhD candidate suggest redundancy of application of dopamine antagonists while gonadolibersins are applied alone, but only via PLGA system ensuring slow release of the hormonal agent what would revolutionize controlled reproduction of many fish species. This is potentially the most important finding and constitutes very important topic 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 my opinion the objectives should be revised while putting focus only on testing PLGA as a hormone delivery system in freshwater fishes since inclusion of one paper, in which the PhD candidate has only 5% contribution does not bring any value to the skillset of the candidate and it is clear that it is beyond the topic of the current Thesis. Besides, I am not in favour of incorporating review article in the thesis what makes the overall structure a bit confusing and makes the introductory part of the document really generic without smooth introduction to the topic. Apart from that, some aspects have been introduced in the General Discussion chapter, what the most probably stems from the fact that PhD candidate realized some information can be missing for the reader (see also overall commentary of mine below). The fact that the PhD candidate wrote the review is - of course - „in plus“ for the candidate and her skillset, but overallly should be avoided to be part of the work. The remaining parts of the Thesis are straightforward and does not create any doubts it is a solid PhD thesis. Overall 5 original research articles being in the core of the thesis is a very good achievement for the PhD thesis. In summary, the PhD Thesis of Jindřiška Knowles meets the criteria and I definitely recommend her candidature to proceed with the process and allow the defense.

OVERALL COMMENTARY ON THE PhD THESIS

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

Chapter 1.1: General Introduction: lack of information on the current state of the knowledge on sustained drug delivery system. I realize that later it is explained in the Chapter 1.2 in the review paper written by PhD candidate and her supervisor, but still this chapter does not provide sufficient information on what the thesis is about. The formulation of the aim of the thesis appears pretty unexpectedly. Consequently, there are some information missing (concise and clear) that the PhD candidate concern will be application of alternative strategy for administration of the hormones in controlled reproduction of fishes bridging the theoretical (written correctly) information on hormonal regulations of ovulation and spermiation and the first review paper being actually the real introduction to the thesis. Besides, the numbering is pretty confusing. The first part in chapter 1.1 entitled „Neuroendocrine regulation of fish reproduction“ should be, in fact chapter 1.1.2, and the subsequent chapter should be 1.1.2.1 since chapter 1.2 is the first paper presented. Both aspects I would not consider as a serious flaw, but this is still pretty confusing making the structure of the thesis for reconsideration.



The next chapter of the thesis is another introductory part where the PhD candidate is having really minor share. This is very technical paper, where encapsulation procedure was being optimized and verified using modern visualization and evaluation tools. In fact, this, together with the first article presented in this thesis could be presented in the form of clear and concise Introduction chapter rather than being part of the thesis, since the first paper is only a review, and the second one being only side-work (important, indeed) of the PhD candidate. In my opinion, these two papers are not really enhancing the quality of the thesis and only create confusion. For example, the PhD candidate is writing, that the aim was to develop innovative method for hormonal stimulation with only 5% contribution to the developmental works. So, in the end the entire aim of the thesis should also be carefully reconsidered with main emphasis put on testing the developed method.

In the next chapter, the PhD candidate - having majority of share – tested the developed hormonal induction system in *Coregonus peled*, highly important commercial species. Generally the paper is well written, though I would like to be informed why the positive control group (mGnRHa) has received half of the dose when comparing to the PLGA-encapsulated hormones. Especially, that in the paper it is not described what was the rationale for using these particular doses. Of course, this does not really affect the final conclusions drawn from this experiment since the Authors did not observe significant differences in relation to mode of hormonal delivery also suggesting that the fish were highly receptive to any kind of hormonal stimulation strategy.

In the next study, where PLGA-encapsulated hormones were applied to reproduction of *Sander lucioperca*, PhD candidate (having 50% of share) has shown that encapsulation of mGnRHa with low dose had the best effects in terms of number of females ovulated. However, it is not really clear to me why only 1 and 4 µg/kg/day were chosen? In the paper the explanation was very scarce: „based on findings of various studies“; without providing the exact studies. The choice of the doses should be provided. Also, I am wondering how much close to the reality is the release of the hormone in *in vitro* medium when compared to *in vivo* testing, where various enzymes are capable of playing role in releasing the encapsulated enzyme? To justify this question I would like to highlight the fact that significant hormonal level after administration of these agents has been observed only 20 h following injection. I would like to ask the PhD candidate to elaborate on that.

Another two chapters were related to the induction of spermiation in northern pike (*Esox lucius* – chapter 5) and sterlet (*Acipenser ruthenus* – chapter 6). Overall, these were state-of-the-art experiments with appropriately chosen methods, though I am also wondering why the Authors have chosen as a positive control gonadotropins rather than GnRH, being encapsulated. While choosing these agents we can not really judge whether the differences observed between experimental and positive control groups were due to the indeed slowed release of the hormones or the mode of action of those two hormonal agents. The last paper, chapter 7, aimed at investigation of PLGA encapsulated GnRH (only 1 dose) in comparison to hCG and GnRH combined with metoclopramide (MET). And this study brings the strongest conclusions, where it is stated that PLGA-encapsulation can overcome dopamine effect in species, in which such additional pharmacological intervention is still needed (by application of dopamine antagonists such as metoclopramide). And this would be a very important, if not



even the most important, finding, if the Authors would introduce additional experimental groups such as single injection of pure GnRH, without MET. I understand, that the main assumption was that this species require application of MET during the hormonal induction made with GnRH, but still the picture is again not so clear. Besides, in neither of the paper the Authors did not make attempts to compare the PLGA with already before tested agents and currently being in use in several fish species, what should be addressed by the PhD candidate during the Thesis defense.

Nevertheless, in overall the concerns and questions of mine should be considered as minor remarks since all the papers have underwent rigorous peer-review in JCR-indexed journals and overallly they are significantly contributing to our knowledge on hormonal induction of ovulation and spermiation in freshwater fishes.

General discussion chapter is overallly concise and clear. However, first two paragraphs should be moved to Introductory part, as it is not actually a discussion. Some other paragraphs are more suitable to methods sections, which in such thesis should simply be omitted (like the one on page 95 starting from „To accurately record...“). Besides, the conclusions drawn are reflecting the outcomes of the papers.

FINAL RECOMMENDATION

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

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Date and place

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Name and signature