



Confidential

Supervisor's Review of USB RIFCH PhD Thesis

Surname of the PhD student: Knowles	Name of supervisor: M.Sc. Peter Podhorec, Ph.D.
Title of PhD thesis: Using of prolonged releasing drug systems in fish reproduction	

OVERALL COMMENTARY ON THE PhD THESIS

Jindřiska Knowles's thesis focused on the improvement of fish artificial reproduction by the method of sustained hormone release from PLGA microparticles. Based on my knowledge this is the first comprehensive study about the successful usage of PLGA microparticles in freshwater aquaculture which underlines the innovativeness of the evaluated thesis.

Over the course of her PhD studies Jindřiska Knowles became familiar with the methods of PLGA microparticles preparation, new methods of artificial fish reproduction, evaluation of egg and sperm qualitative and quantitative characteristics, statistical analyses of obtained results and mastered the technique of sex steroids analysis by enzyme-linked immunosorbent assay method.

Her PhD thesis is composed as a compilation of seven published papers in peer-reviewed IF – journals, starting with the introductory part supplemented by a comprehensive review paper about sustained drug delivery systems in aquaculture followed by research papers focused on the effect of PLGA microparticles with GnRHa on induction of ovulation and spermiation in freshwater fish species. The last chapter is represented by general discussion and relevant conclusions.

I consider the following to be the most significant achievements of Jindřiska's work:

- A) Elucidation of sex steroid surge profiles during the treatment period by PLGA microparticles and standard treatment in various freshwater fish species.
- B) Proof of non-inferiority of administration of mGnRHa encapsulated in PLGA microparticles versus standard treatment on the quality and quantity of the obtained male and female sex products and larvae in any of the tested freshwater fish species.
- C) Successful induction of final oocyte maturation and ovulation by low dosage of mGnRHa releasing from PLGA microparticles leading to significantly better results than those obtained after standard treatments (*Sander lucioperca*).
- D) Successful improvement of spermiation by PLGA microparticles with GnRHa leading to higher



motility percentage (28 s post-activation), significantly increased sperm volume with total sperm count (Balantiocheilos melanopterus) and extended sperm collection (Acipenser ruthenus) compared to standard treatment.

Obtained results provide valuable information about the potential of PLGA microparticles as a system for long-term release of GnRHa which outperforms standard ways of hormone application and gain significantly better results. This knowledge is highly beneficial for the field of sustained drug release and for the practical use of PLGA microparticles in aquaculture.

Overall has Jindriska Knowles published four papers as a first author and four papers as a co-author in peer-reviewed journals with impact factor. In addition to previous she authored two methodologies and co-authored one methodology about her results with PLGA microparticles usage in freshwater aquaculture and this way made it easily accessible to the czech professional public.

As a supervisor of Dipl.-Ing. Jindriska Knowles, I strongly recommend her PhD thesis for defense.

FINAL RECOMMENDATION

- ☒ can be recommended for defence of PhD Thesis
☐ can be recommended with reservations for defence of PhD Thesis
☐ can not be recommended for defence of PhD Thesis

3. 8. 2023, Torre del Mar

Date and place

Podhorec

surname and signature