

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:

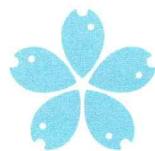
Surname: Socha	Institution: University of Agriculture in Krakow, Poland
Name: Magdalena	
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Please describe your professional relationship to the PhD student: I have no professional relationship to the PhD student	Please describe your field of expertise: neurohormonal regulation of fish reproduction in normal and intoxicated environment

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**):

It should be clearly emphasized that the studies, which the Author focused on, are important for worldwide sustainable aquaculture, especially in the culture of economically important fish species. The obtained results in this PhD thesis, provide valuable information for both basic and applied research. First, it increased the knowledge in the field of sustained drug release and, what is very important, the knowledge for practical use in the aquaculture, improving artificial reproduction, by using an innovative method of hormonal stimulation – mGnRHa encapsulated in PLGA microparticles. This method is suitable for commercially important fish species, eliminates stress connected with repeated hormone injections and allow to use lower GnRHa doses. Furthermore, the use of mGnRHa encapsulated in PLGA microparticles showed no negative effects on the quality of the obtained eggs, sperm and larvae in tested fish species.



Elaboration of the PhD thesis, objectives of the work and deliverables

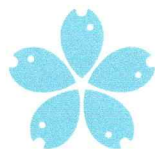
Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (extent ¼ – ½ page):

A main text of this dissertation is well, logically and clearly written in English. A structure of the dissertation is typical and processed according to the requirements guideline for writing the PhD thesis at the FFW USB. The main objectives of this work was to develop and verify an innovative method of hormonal stimulation using PLGA microparticles as a system for sustained release of a synthetic analogue of mGnRH in several economically important fish species and elaborate study reviewing methods of hormonal treatment in fish, and discuss the potential of PLGA microparticles in female and male fish reproduction. All the publications and the obtained results described consecutively in the chapters correspond to aims of the PhD thesis. After the overview about the formation of PLGA microparticles and their physical, chemical, analytical, and pharmaceutical properties (Chapter 2), the next chapters (3, 4) show the impact of PLGA microparticle treatment on the reproductive output of females (ovulation/quality of eggs in peled and pikeperch) of) and males (spermatation/quality and quantity os sperm in northern pike, sterlet and Bala shark – respectively, Chapters 5-7). I would like to underline that the elaboration of the PhD thesis, the presentation of obtained results (important and valuable!), fully correspond to planned objectives.

OVERALL COMMENTARY ON THE PhD THESIS

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

The PhD thesis written by MS Jindřiška Knowles, submitted for evaluation, has been prepared at the Institute of Aquaculture and Protection of Waters, Faculty of Fisheries and Protection of Waters of University of South Bohemia in České Budějovice under the supervision of Mgr. Peter Podhorec Ph.D. The consultant of this work was Prof. Dipl.-Ing. Jan Kouřil, Ph.D. The work, written in English, includes 111 pages and consists of eight chapters: first General Introduction, then chapters 1.2-7 are entitled as attached seven original, very well published papers, and chapter 8 with General Discussion, English and Czech summary, Acknowledgements, List of publications, Training and Supervision Plan of Study and *Curriculum vitae*. It's worth pointing out, that MS Jindřiška Knowles is the first, and leading author of **four presented publications** with mean share about 60% in their formation. These papers were published in: *Veterinarni Medicina*, IF 2020 = 0.565; *Czech Journal of Animal Science*, IF 2020 = 1.267; *Animals*, IF 2021 = 2.752 and *Animal*, IF 2021 = 3.240. In addition, She is a co-author of 3 papers (*Acta Poloniae Pharmaceutica*, IF 2021 = 0.53; *Animals*, IF 2021 = 2.752; *Fishes* IF 2021 = 2.385). Presented PhD thesis contains a large number of valuable



results, about the formation, use, and outcomes of PLGA microparticles as a system for long-term release of GnRH analogue in the reproduction of chosen fish species (peled, pikeperch, northern pike, sterlet and Bala shark). It needs to be highlighted that, this innovative method of hormonal stimulation was the first time studied in fish. Therefore, the Author focused on the establishing the optimal dose of mGnRHa encapsulated in PLGA microparticles, evaluating the level of sex steroids (E2, T, 11-KT) after the stimulation of the broodstock and estimating the quality and quantity of obtained eggs and sperm. It should be noted that very interesting results was observed in pikeperch females, in which a low dose of mGnRHa encapsulated in microparticles induced ovulation, what may lower costs of artificial reproduction in this species. Next, very important findings is no negative effects of PLGA microparticles on broodstock (no adverse effect on quality of obtained sex products) and offspring (no abnormalities in hatched larvae). Another advantage of the use of hormone (*inter alia* mGnRHa) encapsulated in PLGA microparticles is elimination of repeated injections, meaning less stress to spawners. Conducting the studies, at a high scientific level, required from the Candidate the use of a variety of experimental procedures, different research techniques, as well as enormous amount of laboratory work. It should also be underlined that MS Jindřiška Knowles has confirmed an extensive knowledge of the scientific literature on the subject of research, especially on fish reproduction.

In my opinion presented PhD thesis, written by MS Jindřiška Knowles entitled: "Using of prolonged releasing drug systems in fish reproduction" **is of high scientific significance and value. Therefore, I recommend this PhD thesis for defence.**

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

29th June 2023, Kraków, Poland

Socha Magdalena