

Jihočeská univerzita v Českých Budějovicích University of South Bohemia in České Budějovice Czech Republic

Confidential

Supervisor's Review of USB RIFCH PhD Thesis

Surname of the PhD student: Vitaliy Kholodnyy	Name of supervisor: Sergii Boryshpolets
Title of PhD thesis: Sperm/egg interaction in freshwater fish: influence of environment on fertilization	
process	

OVERALL COMMENTARY ON THE PhD THESIS

Vitaliy Kholodnyy joined laboratory of reproductive physiology as a PhD student in 2016. At that time, he has already had PhD degree in "Cryobiology" and worked in the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences in Ukraine. His decision to move to another country in order to pursue scientific career in a new place and a new field, in spite of associated complications, characterizes him as a purposeful and dedicated to science person.

Vitaliy started his research in the area of fish reproduction with a very wide and complex question about how does the fertilization occur in fresh water? After a deep and accurate literature search, main hypothesis and experimental plan were built and used as a background for his study (summarized in Chapter 1). In this chapter, possible processes of sperm/egg interaction and the effect of environment on them are discussed, predicting species-specific mechanisms of gametes interaction. Moreover, it is suggested that these mechanisms depend on a strategy of reproduction, which were modified in parallel as a function of time. To verify the hypothesis, three different freshwater fish species with different reproduction performance and spermatozoa physiology were chosen for the study. The data obtained are presented in the following chapters: rainbow trout (Chapter 2), sterlet (Chapter 3) and carp (Chapter 4). Due to the previous experience and knowledge, Vitaliy quickly learned the basic methods and techniques, used in the lab. It must be noted that to solve the objectives of his study, the experiments should have been be more complex than regular laboratory techniques, and they required a special approach for each fish species and each specific case. During his study, Vitaliy learned, adapted and elaborated several techniques for analyses of sperm motility, sperm response to stimuli, sperm accumulation, fractionation of ovarian fluid and different fertilization assays, which helped him to reach the results and progress in the study. In addition to other techniques, Vitaliy learned a specific method of intracellular Ca imaging (during his stay at Marine station in Japan under supervision of Prof. Manabu Yoshida). In conclusion, Vitaliy managed to collect and process a lot of new and interesting data about sperm/egg interaction in three freshwater fish species and to correlate them with their reproduction strategy (results are summarized in Chapter 5). His activity and results open an absolutely new and very promising direction for further research in the field of fish reproduction, which potentially may contribute to understanding of rules of different reproduction strategies in general and help to optimize artificial reproduction in selected species in particular.

Besides work, I am also happy to have Vitaliy as a colleague in our lab because of his enthusiastic and friendly personality. During this time, he became a part of our team.

Finally, I strongly recommend the thesis of Vitaliy Kholodnyy for defence.

FINAL RECOMMENDATION

can be recommended for defence of P	hD Thesis
can be recommended with reservations for defence of PhD Thesis	
can not be recommended for defence	of PhD Thesis
Vodinary 07.07.2020	
Date and place	surname and signature