



Confidential

Supervisor's Review of USB RIFCH PhD Thesis

Surname of the PhD student: Roman Franěk	Name of supervisor: Martin Pšenička
Title of PhD thesis: Germ cell manipulations as a tool to manage and produce isogenic lines in fish	

OVERALL COMMENTARY ON THE PhD THESIS

Roman joined my laboratory in 2015 after finishing master study program of fishery at our faculty. His previous knowledge about fish allowed to him adapt quickly and he started to work independently although his previous topics were mainly focused on fish toxicology and disease. Experienced researchers know very well that isogenic line production in fish is extremely difficult and sometimes it is more close to impossible rather than possible. Roman tried to take a maximum from germ cell manipulation techniques and attempted to develop a novel way for isogenic line production and maintenance utilizing chromosome manipulation, germ cell cryopreservation and transplantation. Two papers published in Q1 and Q2 journals are describing development of cryopreservation protocols for common carp spermatogonia and oogonia. I strongly appreciate that both studies ended with transplantation trials of cryopreserved germ cells into surrogates, although it took additional months of work to finalize the experiments. I am looking forward that presented work on germ cell cryopreservation in common carp will be once recognized by aquaculture and will facilitate to real needs of genetic resources banking.

Remaining chapters in the thesis are consisted of one review article which has recently received major revision in Reviews in Aquaculture journal. Second paper about extensive validation of zebrafish triploid surrogates is currently under review in Theriogenology (Q1). Yet not submitted manuscripts are presented in chapter 5 and 7. Manuscript regarding our attempts for isogenic gamete production from goldfish surrogates is summarizing our long term work where thousands of goldfish recipient were transplanted with germ cells of common carp. Unfortunately, we are in the middle when we reported production donor-derived carp sperm from goldfish, while still waiting for carp eggs. However, I strongly believe that we will succeed soon as only fraction of recipients matured so far. Second unpublished manuscript is describing a novel way for germline chimera production using intraperitoneally grafted blastomeres in zebrafish. I would like to outline importance of this method, because we are going to use it in other experiments and I personally expect publication in a prestigious journal. We are also really proud of this method because nowadays it is being currently applied by our Japanese colleagues for surrogate reproduction in loach and tuna.

Roman has been involved not only in experiments presented in his thesis. He is co-author of two papers. He also actively participated in yet unpublished experiments of his colleagues. Moreover, he is in charge of zebrafish colony and providing assistance to several colleagues who are coming to our laboratory to perform experiments. During his PhD and internships (INRA, Rennes, France and Hokaido University in Japan) he gained experience with other fish species such as sturgeons, tench, crucian carp, loach and rainbow trout. His wide range of interest is apparent from number of presentation at conferences.

I appreciate Roman for his active participation during projects preparation. He received two year GAJU



project, nowadays he is leader of Technologic Agency project. Beside this, he has been involved in two TNA project preparation. Results of the presented thesis have been several times presented at international conferences. Roman also participated at two annual meeting of Aquaexcel2020 project presenting our progress with isogenic carp.

Finally, I am pleased to express my satisfaction with Roman during his PhD. He is very dedicated, open minded and able to develop ideas and perform experiments independently. I believe that we will be able once perform all experiments which we think out. I strongly recommend his 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

9.5.2019 in Vodňany

Date and place

PŠENIČKA

surname and signature