



Confidential

Supervisor's Review of USB RIFCH PhD Thesis

Surname of the PhD student: Shivaramu Sahana, MSc.	Name of supervisor: prof. Ing. Martin Flajšhans, Dr. rer. agr.
Title of PhD thesis: Hybridization of sturgeons	

OVERALL COMMENTARY ON THE PhD THESIS

Sahana Shivaramu entered my lab in autumn 2015 as a Master of Fisheries Science. She was also accepted as so-called Early Stage Researcher (ESR) in a project "Improved production strategies for endangered freshwater species (IMPRESS)" No. 642893 of Marie Skłodowska-Curie Innovative Training Network programme financed by the EU within Horizon 2020 framework. Her task was to study the problems of sturgeon hybridization not only for pure aquaculture, but also from the point of view of innovative production strategies for the conservation and management of endangered freshwater fish species in the IMPRESS project.

Step by step, Sahana became familiar with the system of sturgeon breeding, techniques of artificial reproduction, egg incubation and progeny nursing; with design, management and operating the fitness tests; with fish tagging, sampling and molecular genetic analyses of microsatellite markers for sterlet population divergence and assessing the effects of heterozygosity and level of polymorphism on fitness of hybrid/purebred families. Moreover, she extended her initiative to learn more on population genetics and genomics within the IMPRESS project fellowship. She spent the mandatory 3 months of stay abroad, altogether at the Leibniz-Institut für Gewässerökologie und Binnenfischerei in Berlin, Germany during her experiment with critical swimming speed of sterlet families, in ZF-Screens at the university of Leiden, the Netherlands on genomics and in Aqvaforsk Genetics, Sunndalsøra, Norway where she learned more on population genetics. During her studies, she also completed all the due courses for PhD students, as well as five training schools for all the ESRs within the IMPRESS ITN. She participated in three international symposia in Europe, presenting two lectures and one poster on the topic.

Sahana Shivaramu proved her pedagogical abilities as supervisor of two summer school students (2016, 2018) and, within the IMPRESS project, in field- and lab training of ESRs during the respective training schools and in public dissemination program at a Norwegian high school in Arendal.



Her PhD thesis is conducted as a compilation of two papers published in peer-reviewed IF – journals (Chapters 2 and 4), with the introductory review on the problems of sturgeon conservation, reproduction, hybridization and genetic tools to study it (Chapter 1), further accompanied with ready-to-submit manuscripts on genetic analysis and performance tests of intraspecific hybrids of Danube and Volga sterlets (Chapter 3) and on heterosis estimates for growth and survival traits in sturgeon purebreds and interspecific hybrids (Chapter 5). The last Chapter 6 deals with general discussion which attempts to summarize the present scenario of sturgeon hybridisation(s), genetic tools to monitor hybrids and, last but not least the implications for sturgeon fisheries management.

I can see the most important milestones of progress achieved in work and thesis of Sahana Shivaramu as follows:

- 1) The analysis of the genetic pattern in the parental broodstock of sterlet from Danube and Volga river basin populations (fixation index values) confirmed the population divergence between Volga and Danube stocks. Microsatellite markers enabled to successfully assign the parentage and further helped in ascertaining the broodstock with high genetic diversity.
- 2) The Danube (♀) × Volga (♂) hybrids displayed higher genetic polymorphism and performed better than purebred crosses in growth trials whilst the reciprocal hybrid exhibited intermediate values. Significant differences were recorded between reciprocal hybrids in growth traits which can be valuable for aquaculture. The obtained results showed no genetic incompatibilities (e.g. breakdown in the mean number of alleles in hybrids compared to purebreds) in the first hybrid generation.
- 3) Comparing intraspecific hybrid crosses with purebred crosses, no difference in swimming performance was observed and swimming performance was shown to rather be a family specific trait.
- 4) Interspecific hybridization of two evolutionary octaploid species, Russian- and Siberian sturgeon showed the hybrids exhibited the highest weight growth and/or cumulative survival but it was not supported by finding any significant differences in heterozygosity level among the families studied.
- 5) Interspecific hybridization of evolutionary tetraploid sterlet with evolutionary octaploid Siberian sturgeon and vice versa showed sturgeon hybrids had higher mean body weight compared to their purebreds, with significantly higher mean number of alleles, and positive heterosis was recorded for



mean body weight of sterlet x Siberian hybrid throughout the assessment periods.

6) Results confirmed that improvements in fitness-related properties of sturgeon intra- and interspecific hybrids cannot be taken all alone. It is always necessary to take into account not only the production of hybrid but also the position of individual species and population in a hybridization matrix.

7) Potential risks of hybrids' use in aquaculture in case of their release/escape into open waters for genetic integrity of wild pure species/ populations were summarized as well.

As a supervisor of Sahana Shivaramu, MSc., I can state that the aims of this PhD thesis were fulfilled and that Sahana came out with few novel findings which enriched the yet fragmented knowledge on sturgeon hybridisation.

Finally, I 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

May 20, 2019 Vodňany
Date and place

prof. Ing. Martin Flajšhans, Dr. rer. agr.
surname and signature