



Fakulta rybnářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

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

Confidential

Review of USB FFW PhD Thesis

First name(s), surname, titles of the PhD student: Sahana Shivaramu, M.Sc.	First name(s), surname, titles of supervisor: Prof. Dipl.-Ing. Martin Flajšhans, Dr. rer. agr.
Title of PhD thesis: Hybridization of Sturgeons	
REVIEWER:	
Surname: Kohlmann	Institution: IGB Berlin Germany
Name: Klaus	
Titles: Dr. agr.	E-mail: kohlmann@igb-berlin.de
Please describe your professional relationship to the PhD student: I had and have no direct professional relationship to the PhD student. However, I reviewed one of her manuscripts that was published in the Czech Journal of Animal Science later on and included in the thesis as chapter 4.	Please describe your field of expertise: Fish genetics with focus on population genetics, and development and application of nuclear and mitochondrial DNA markers.

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**):

With the exception of occasional records of naturally occurring hybrids, or survival and growth of artificially produced hybrids for aquaculture purposes – consequences of hybridization of sturgeons were only poorly studied if at all up to now. However, this information is urgently needed and to gather it has high priority, in particular for species that are critically endangered and/or facing the risk of extinction such as most of the sturgeons. The present thesis focusses on fitness related traits of reciprocal intraspecific hybrids between sterlet sturgeon originating from the Volga and Danube rivers, and reciprocal interspecific hybrids between Siberian and Russian sturgeon (species of the same ploidy level, their hybrids are probably fertile), and Siberian and sterlet sturgeon (species of



different ploidy levels, their hybrids assumed to be sterile) in comparison to their pure parental species. Thus, it represents a valuable contribution to close the knowledge gaps mentioned above. The obtained results are original, competitive at the international level of sturgeon research and of high scientific importance for sturgeon species conservation measures, in particular for the management of broodstocks and the establishment of breeding schemes in the context of supportive stocking programs, but also for the utilization of hybrids in the commercial aquaculture. For details see overall comments.

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**):

The main text of chapters 2 to 5 (two published papers and two manuscripts) is well structured according to general rules of scientific publications. Also, the ordering of these chapters from intra- to interspecific hybridization is logical. The selected methodological approaches are appropriate to achieve the objectives of the thesis. In contrast, although being very extensive, the General Introduction (chapter 1) is lacking such a clear and logical structuring. It could have been shorter due to overlaps with the Introductions of the published papers (chapters 2 and 4) and manuscripts (chapters 3 and 5). Moreover, this chapter contains a few minor flaws. Large parts of General Discussion (chapter 6) rather represent an Introduction whereas recommendations for future research and management implications are too short. For details see overall comments.

The main part of the thesis consists of two publications in international, peer-reviewed journals (chapter 2: Journal of Applied Ichthyology, IF 2018 = 0.877; chapter 4: Czech Journal of Animal Science, IF 2018 = 1.008) and two manuscripts (chapters 3 and 5, without information if and to which journal submitted). The described results correspond to the objectives of the thesis as outlined at the end of General Introduction (chapter 1).



OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The topic of the thesis – hybridization of sturgeons – has two important aspects: (1) In nature intra- and interspecific hybrids may originate from mating of native individuals with accidental aquaculture escapees or individuals from non-native populations or species used for restocking purposes. Both scenarios would have severe negative impacts on the genetic integrity of the pure populations and species. (2) In aquaculture intra- and interspecific hybrids are produced to exploit possible heterosis effects mainly for growth and survival. Additionally, sturgeon broodstocks are kept for offspring production in supportive stocking programs. Therefore, information on the fitness of sturgeon hybrids is urgently needed to allow a sound estimation of the threat they pose to wild sturgeon populations and species as well as of the benefits they might provide to sturgeon farmers. The thesis investigated both intra- and interspecific hybridization and their consequences on fitness related traits using fertilization and hatching rates, critical swimming speed, growth and survival, and microsatellite DNA polymorphisms of reciprocal crosses in comparison to pure parental populations/species as indicators.

Chapter 1: general introduction

This chapter is very extensive covering all aspects of sturgeon biology, ecology, evolution and genetics relevant to the topic of the thesis – natural and artificial hybridization. However, the order of paragraphs is not always logical; topics are sometimes disorganized. For example: sturgeon systematic classification is followed by survival threats and conservation actions, then back to sturgeon reproduction followed by a general paragraph on hybridization in fishes, then back again to sturgeon (genome evolution, polyploidy, hybridization and genetics). Likewise, a logical order of sturgeon species is missing in Table 1. Moreover, in Table 1 the three *Pseudoscaphirhynchus* species are duplicated. The classification of hybrids contains a mistake: *Carassius x Cyprinus* is an intergeneric hybrid, not interspecific as stated. The heading of paragraph 1.6.1 (Application of microsatellites ...) is slightly misleading since large parts describe other genetic markers as well. The same structuring number 1.7 is used for Aims of the thesis and for References.

Chapter 2: critical swimming speed of intraspecific sterlet sturgeon hybrids

This chapter is a reprint of a paper published in the *Journal of Applied Ichthyology* with the PhD student's share of work of about 60%. Intraspecific crosses of sterlet from the Danube and Volga rivers were performed to investigate the effect of hybridization on the swimming performance of juveniles. Swimming performance is an important indicator of the potential for survival of fishes, their ability to access habitats, avoid predators and acquire food. The reciprocal hybrids did not differ significantly from the pure parental populations, neither under constant temperature nor under a regime mimicking natural temperature variations.

Chapter 3: genetic analysis and performance tests of intraspecific hybrids of Danube and Volga sterlets

This chapter is a manuscript with the PhD student's share of work of about 40%. The same experimental design as reported in chapter 2 was used to investigate the effect of hybridization on



fertilization and hatching rates as well as cumulative survival and body weight up to 504 days post hatch. Since one of the hybrids (Danube x Volga) showed the highest average body weight and the reciprocal hybrid (Volga x Danube) the highest survival in comparison to the parental populations it is concluded that intraspecific hybrids could have advantages in commercial aquaculture. Future research focusing on the fecundity and reproductive fitness of F1 hybrids as well as fitness-related traits in F2, backcrosses and subsequent generations is recommended in the thesis.

Chapter 4: interspecific hybridization of Siberian and Russian sturgeon

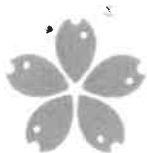
This chapter is a reprint of a paper published in the Czech Journal of Animal Science with the PhD student's share of work of about 60%. This study was specifically designed to investigate the effect of interspecific hybridization of species with the same ploidy level (Siberian and Russian sturgeon) on fitness-related traits. At day 913 post hatch, the Russian x Siberian hybrid reached the highest body weight and the reciprocal Siberian x Russian hybrid showed the highest cumulative survival. Heterozygosity levels of the studied microsatellite loci were similar among all experimental groups. Since these hybrid types might be fertile, it is strongly recommended to prevent their release from hatcheries into the wild. If used for commercial sturgeon aquaculture, the position of the individual species in the hybridization matrix should be considered because of the observed differences between the reciprocal crosses.

Chapter 5: heterosis estimates for growth and survival traits in sturgeon purebreds and interspecific hybrids

This chapter is a manuscript with the PhD student's share of work of about 35%. This study was specifically designed to estimate heterosis effects for growth and survival of interspecific hybrids between species with different ploidy levels (Siberian and sterlet sturgeon). At day 862 post hatch the highest body weight was recorded in the sterlet x Siberian hybrid corresponding to the highest observed positive heterosis. In contrast, the cumulative survival was highest in the Siberian purebred. The mean number of alleles at the studied microsatellite loci was significantly higher in both hybrid groups compared to pure sterlet sturgeons. It is concluded that the studied hybrids could be potentially used in the commercial sturgeon aquaculture to exploit the heterosis effect for growth. However, due to the observed differences between the reciprocal crosses the position of the individual species in the hybridization matrix has to be considered.

Chapter 6: General Discussion

Large parts of this chapter have the character of an introduction or description of obtained results rather than their interpretation and discussion in the context of what was already known and what is new. A part of the first paragraph on intraspecific hybridization on p. 84 (starting with "On the other hand ...") is even almost identical with the paragraph in the middle of p. 18 of General Introduction about consequences of hybridization. On the other hand, the recommendations for future research directions and implications for sturgeon fisheries management and species conservation could have been described and discussed in more detail.



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FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence**
- PhD Thesis can be recommended with reservations for defence**
- PhD Thesis cannot be recommended for defence**

...01.07.2019, Berlin...
Date and place


...Dr. Klaus Kohlmann...
Name and signature



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Sahana Shivaramu, M.Sc.	First name(s), surname, titles of supervisor: Prof. Dipl.-Ing. Martin Flajšhans, Dr. rer. agr.
Title of PhD thesis: Hybridization of Sturgeons	

REVIEWER:

Surname: Kalous	Institution: Czech University of Life Sciences Prague, Faculty of Agrobiolology, Food and Natural Resources, Department of Zoology and Fisheries
Name: Lukáš	E-mail: kalous@af.czu.cz
Titles: prof. Ing., Ph.D.	
Please describe your professional relationship to the PhD student: None	Please describe your field of expertise: Sustainable aquaculture, Ichthyology, Invasive species

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**):

Presented Ph.D. thesis is focused on tracking fitness-related features of interspecific and intraspecific hybrids of sturgeons and their comparison with the characteristics of pure parental species/spatially separated populations.

The results showed that hybrids can have considerable potential for aquaculture breeds, like increasing their survival rate and growth performance. This study brings the first observation of the effect of different types of hybridization on sturgeon fitness-related traits. The presented results are an important contribution to basic research and the perspective of the implementation of the presented findings in practice (evaluation of applied research) is high (aquaculture production). The Ph.D. thesis approved its scientific importance by the acceptance of the presented ideas in enclosed articles in internationally recognized scientific journals (2 published articles). Other 3 manuscripts are of good quality - ready to be submitted or already submitted.



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):

The text of the Ph.D. thesis is of good quality although the author did not avoid some mistakes. In general, the text is clearly written by the standard English language. All chapters are logically built and easy to follow. The introduction is of a larger extent but I appreciate the overall comprehensibility of text regarding the complex issue of hybridization. The selected methodological approaches are standardly used and appropriately chosen to handle with the stated objective. Presented two publications and 3 manuscripts fit well to the defined Ph.D. topic and correspond to four stated aims.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Sturgeons are enigmatic fish, which attract attention due to their strange look, ancient origin, big size, long migrations and not least for their old and unfortunate relationship with humankind. Discovering an enormous number of chromosomes in sturgeon cells and the ability of interspecific and intergeneric hybridization among the family Acipenseridae opened new horizons for a number of experiments. Several of those experiments are part of the presented Ph.D. thesis including swimming performance, grow traits, average weight, cumulative survival and reproductive parameters, all in regards to the various hybrid combination.

Most of the finding could be a promising beginning for its implementation to aquaculture technologies for sturgeon production. The important finding is that it is not just a “blind” production of hybrids but it is always necessary to take into consideration the position of the individual population in a hybridization matrix. Additionally, the work touches the hybridization as a potential problem for biodiversity especially when hybrid offspring is fertile.

I do agree with various arguments regarding the risk of hybridization on native sturgeon populations. But I do not agree with the argument that due to competition between hybrid and non-hybrid sturgeons for food in the wild, the native sturgeons (non-hybrid) could be in the risk of extinction. In such a low abundance as we face today, the competition for food resources is negligible.

I would like to ask regarding the possible effect of elevated homozygosity in pure species. Could this be caused by a dramatic reduction of some local broodstock in the wild? Could be also the homozygosity affected by restocking program? How we should look at heterozygosity in case of such impressive chromosome numbers?

The last question and rather targeted for the general discussion is your opinion about the future destiny of sturgeons?

After a successful defence, I recommend awarding Sahana Shivaramu by title philosophiæ doctor in abbreviation Ph.D.



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FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence
 PhD Thesis can be recommended with reservations for defence
 PhD Thesis can not be recommended for defence

11.7.2019 ÚNĚTICE

.....
Date and place

LUKÁŠ KALOUS

.....
Name and signature