



Fakulta rybnářství  
a ochrany vod

Faculty of Fisheries  
and Aquaculture  
in Vodňany

Jihočeská univerzita  
v Českých Budějovicích

University of South Bohemia  
in České Budějovice  
Faculty of Fisheries

**Confidential**

### Review of USB FFPW PhD Thesis

<b>First name(s), surname, titles of the PhD student:</b> Oleksandr Malinovskiy, M.Sc.	<b>First name(s), surname, titles of supervisor:</b> Assoc. Prof. Dipl.-Ing. Tomáš Polícar, Ph.D.
<b>Title of PhD thesis:</b> Broodstock management of pikeperch ( <i>Sander lucioperca</i> L.) and its effect on eggs and larval production	
<b>REVIEWER:</b>	
<b>Surname:</b> Ljubobratović	<b>Institution:</b> NARIC Fisheries and Aquaculture Institute HAKI Szarvas Hungary
<b>Name:</b> Uroš	<b>E-mail:</b> <a href="mailto:u.ljubobratovic@gmail.com">u.ljubobratovic@gmail.com</a>
<b>Titles:</b> Dr.	
<b>Please describe your professional relationship to the PhD student:</b> colleague, he trained artificial reproduction of pikeperch under my supervision.	<b>Please describe your field of expertise:</b> Pikeperch culture with special emphasis on reproductive biology, spawning induction in controlled conditions, artificial reproduction and larviculture in RAS

### QUESTIONNAIRE

#### ***Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research***

Three out of five presented experimental chapters and its respective publications I find rather eligible for the improvement of the welfare of extensively produced brood fish during the spawning on nests. These publications are indeed competitive and they are touching the rather sensitive part of the culture, such is the reproduction, as well as the type of the broodfish, such are the extensively produced. The nest type of reproduction is yet rather practiced technology all over the Europe and extensively reared breeders are perhaps the most common sources of brood fish in countries such are Hungary, Czech Republic and Poland. Therefore, it involved rather important points of present status of pikeperch culture and it speaks about the improvement of welfare and reduction of mortality which I still find one of the crucial moments of the fish reproduction in captivity. This was rear subject of research in case of pikeperch and thus is on the high level of the general state of the research field in Europe.

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**):



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

After reading the thesis, my main impression is that the majority of the results are very competitive as well as the publications within, however, the title and objectives I find poorly defined and it reduces the quality of the overall structure. My main impression is that the title is too wide for any of the PhD thesis and the present thesis mainly speaks about one short and crucial part of the broodstock management, the spawning, and it clearly targets the welfare of the broodfish. This point is very important and is somehow left out from both title and objectives. The eggs and larvae quality are mentioned in both title and the objectives while it is rather poorly elaborated throughout the thesis and I see no place nor sense for this parameter in this thesis. Two chapters are totally unnecessary in the whole thesis, chapters 4 and 6. Its presence is reducing the quality of the thesis and misleads the reader from the point which should be main objectives of the thesis: welfare of the broodfish. Therefore, in order to improve the quality of the thesis and above all its structure, recommendations are:

1. Redefine the objectives of the thesis;
2. Restructure the introduction part accordingly;
3. Remove chapters 4 and 6 from the thesis;
4. Stronger conclude the most important outcomes of the thesis: what spawning manipulation does the thesis suggest in order to improve the welfare of the broodfish.

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  $\frac{1}{4}$  –  $\frac{1}{2}$  page):

**OVERALL COMMENTARY ON THE PhD THESIS**

Please write comments in extent of 1-2 pages:

The majority of the results presented in the thesis are of high quality indeed leading to the improvement of the welfare of brood fish in captivity. There are however some issues which are leading to reduction of its potential to become high quality thesis which I will present here in the form of comments on each chapter:

1. Introduction

Writing level is poor, especially with regards to presented writing potential of the candidate throughout the experimental chapters. It needs some work in order to reach the level of excellence, which should follow this work and the candidate. Many chapters are written without focus, some of them not on the place while the literature is not always cited in the proper manner.





Therefore, I recommend this part to be fully rewritten and to be more focused on the main issue of the successful trials: extensively reared broodstock; nest spawning; improvement of the welfare of the fish. In order to increase its points, it should rather speak more about similar issues in the culture of similar fish of high economical importance (i.e. seabass) not involving the whole issue of pikeperch broodstock management in several different systems. Main recommendation is to increase the main focus on the most important objectives of the study and elaborate it accordingly. Specific comments are provided in the pdf document attached. Objectives should be reformed, strong attention paid on the overall welfare of the broodfish during and after spawning.

2. The substrate selection and spawning behaviour of pikeperch *Sander lucioperca* L. broodstock under pond conditions.

This chapter is of high quality, no need for changes.

3. Behavior and physiological status of pond-cultured pikeperch (*Sander lucioperca*) broodstock effected by sexual interactions throughout semi- artificial reproduction

As well, high quality chapter and results presented.

4. Sperm quality in pikeperch *Sander lucioperca*: effects of multiple semen collecting techniques, and in vitro storage

Two methodological problems are following this study: wrong method of catheterisation and very short period of evaluation of the multiple semen collection (8 hours instead of several days/weeks). Furthermore, the thesis loses focus with this study, it appears as it is not the place for this study inside the thesis. With respect to the mentioned, I suggest to remove the study from the thesis.

5. Post-spawning bath treatments to reduce morbidity and mortality of pond- cultured pikeperch (*Sander lucioperca* L.) broodstock

High quality part of the thesis, very important outcomes again speaking about the spawning and post-spawning issues and broodstock welfare.

6. Effects of sub-optimal water temperatures on the feeding activity of pikeperch (*Sander lucioperca*) with regards to predator's sex and prey size

I see no point of this study in this thesis. It again removes the focus and is done under the conditions not indeed characteristic for the extensively reared broodstock, fully controlled conditions. If this would be the case, prior to study fish should have been acclimatized to the new conditions and it should have taken part much earlier, during the summer phase. The conclusions of such study are hardly implementable in the practice. It repeats its importance for the management of natural water bodies, however I see not this as objective of the thesis. In order to



improve the quality of the thesis, I suggest to remove this chapter from the study.

#### 7. General discussion

Better writing than general introduction, yet needs some restructuring and a bit stronger definition of the main outcomes and its straightforward implementation in the practice such as:

1. recommend the type of the spawning substrate;
2. recommend the specific steps of the fish manipulation during the spawning;
3. Recommend the procedure of fish manipulation after the spawning.

These conclusions are indeed very important for the culture and are the true quality of the thesis which should be highly emphasized.

My final recommendation is to defend the thesis after addressing the suggested points. I find the quality of the majority of presented results high and on the level of a decent PhD thesis, finally recommending the candidate to be allowed on the possibility to graduate.

Sincerely,

Uros Ljubobratovic.



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in České Budějovice -  
Czech Republic

**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

1<sup>st</sup> July 2019, Szarvas, Hungary

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Date and place

Uros Ljubobratovic

.....

Name and signature





**Confidential**

**Review of USB FFPW PhD Thesis**

<b>First name(s), surname, titles of the PhD student:</b> Oleksandr Malinovskiy, M.Sc.	<b>First name(s), surname, titles of supervisor:</b> Assoc. Prof. Dipl.-Ing. Tomáš Polícar, Ph.D.
<b>Title of PhD thesis:</b> Broodstock management of pikeperch ( <i>Sander lucioperca</i> L.) and its effect on eggs and larval production	

**REVIEWER:**

<b>Surname:</b> Zarski	<b>Institution:</b> Institute of Animal Reproduction and Food Research of Polish Academy of Science Olsztyn
<b>Name:</b> Daniel	
<b>Titles:</b> Dr.	<b>E-mail:</b> danielzarski@interia.pl
<b>Please describe your professional relationship to the PhD student:</b> I met PhD student several times while visiting the laboratories of USB FFPW as well during the conferences. PhD candidate was also a trainee of mine during the workshop on controlled reproduction of pikeperch (organized in Belgium in September 2017).	<b>Please describe your field of expertise:</b> My scientific interest involves controlled reproduction and larviculture of freshwater fish species, with specialization on percid fishes (including pikeperch). My research expertise involves reproductive protocols, evaluation of gametes quality as well as factors conditioning gamete quality. In my work I am addressing the research problematics through evaluation of zootechnical indices as well as molecular tools (including transcriptomics and proteomics).

**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**):

The PhD thesis refer to a very important aspect of aquacultural production (regardless the target of such production – restocking or commercial ongrowing) which is broodstock management during the spawning operation of highly valued fish species - pikeperch. Generally, the Thesis includes original research ventures not considered by the other authors before. The data presented in this Thesis has high scientific importance, in my opinion. The experiments performed and described refer to a very narrow part of aquaculture-related



science, which are the specific protocols applicable to pond-reared pikeperch breeders, being very specific research object. These problems are currently tackled mostly by only three countries – Czech Republic, Hungary and Poland (the latter to a lesser extent). This makes the overall value of the scientific findings presented high, especially that they are novel contributions to our knowledge. Currently pikeperch aquaculture and management of the wild stocks is of highest concern addressed by several distinguished research groups across Europe. Therefore, the Thesis may have significant impact on the future development and/or expansion of the production of juveniles of pikeperch with the use of pond-reared broodstock. This would bring more and more attention of the researchers in Central and Eastern Europe, being the region potentially benefiting the most from the data presented due to the suitable pond infrastructure. In my opinion, effective handling protocols of pikeperch – being highly stress-sensitive fish – addressed by this Thesis is the crucial element allowing to convince the pond-aquaculturists toward diversification of their production where the pikeperch would potentially constitute an important part of the entire production. So, in sum, there are high perspectives of using the results of the Thesis by researchers and aquaculturists.

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#### ***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 thesis is composed of 7 Chapters. Chapter 1 is an introductory description to the problematics tackled by the PhD candidate in his Thesis. Chapters 2-6 are containing the research papers or manuscripts of the articles being the main scientific content. The Thesis is closed with general discussion and other appropriate parts constituting Chapter 7. In overall, the Thesis is structured appropriately and logically. The PhD candidate outlined the most important aspects related to the topic he was working on. In the introductory Chapter the role of pikeperch in the aquaculture sector was described which was then followed by the description of reproductive biology of this species. In further parts the PhD candidate was aiming to refer to a more specific topics such as controlled reproduction and current problems in broodstock management closing this Chapter with description of the goals of his Thesis. And overall, the concept and structure of the introductory part is suitable for the thesis on broodstock management. However, one must be pointed out that in this part there was lack of definitions allowing to understand what the “broodstock management” actually is, in his point of view. Besides, I am missing in this part the information on significance of using pond-reared fish in aquaculture and other potential applications. In this sense, the PhD candidate did not enough clearly explained the area of research being involved in (spawning of pond-reared fish; production of juveniles coming from pond-reared fish) as well as not enough justified the problems tackled by his research. Nonetheless, I have no doubts that most of the research undertaken in this Thesis was worth of investigation. However, the lack of focus on the main topic of the thesis (broodstock management) need to be pointed out.

The thesis – according to the title – should focus on the broodstock management (here not defined, unfortunately) and its effect on the egg and larval production. However, none of the paper (Chapters 2-6) is actually referring to the effect of variable tested to the effectiveness of reproduction. Only in Chapter 2 the eggs were described to be obtained and some scarce data on their quality was presented without any further effect on larval performance. So, in effect – despite the title suggest the author was investigating the effect of the broodstock management on eggs and larvae – there is no information on how the aspects studied affected the spawning effectiveness. Besides the objectives were defined properly, the title of the thesis and the justification

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part of the Thesis does not correspond to each other and to the title of the entire Thesis.

In sum – the lack of definition of what the broodstock management is (or more precisely – how it was considered in this Thesis) and the lack of consistency between the title of the Thesis, justification of the scientific problem undertaken and the objectives defined are the main concerns of mine. However, in my opinion this can be easily adjusted by suitable revision made to the title and the whole Chapter 1 of the thesis.

### **OVERALL COMMENTARY ON THE PhD THESIS**

**Please write comments in extent of 1-2 pages:**

The Thesis of Oleksandr Malinovskiy is interesting scientific venture in the very specific field of propagation of pond-reared broodstock. The PhD candidate, working with a highly valued commercial fish species, in his work has focused on the problematics related to handling the fish before and after the spawning operation as well as investigated the aspects of how these manipulations affect the physiological status of the fish. And the overall topic as well as the most of the research included in the thesis are solid piece of experimental work. However, several meritoric inconsistencies paid my attention which I must refer to in my review.

Firstly, I need to point out that in my opinion the title of the Thesis is misleading, when comparing it to the content of the entire Thesis. The title refer to the aspects of broodstock management and it's effect on egg and larval production whereas we can read from Chapters 2-6 that only the aspect of different handling protocols were evaluated from the perspectives of their harmfulness to the fish. Only in the Chapter 2 the PhD candidate briefly refer to the overall spawning performance where hatching rate of the larvae obtained was evaluated. Besides, Chapter 4 refer exclusively to the aspect of multiple sperm collection and the effect of this procedure on sperm – really theoretically related to the egg and larvae production. Therefore, I suggest that the title should be changed accordingly: (1) the information about the effect on egg and larval production should be removed and (2) information on the fact that the Thesis is about the management of „pond-reared broodstock during the reproduction operation“ should be introduced. This would allow to avoid further critics on inconsistency between the title and the content of the Thesis.

The Introductory and General Discussion parts indicates that PhD candidate acquired suitable skill in scientific writing and using the scientific literature while reporting the data. Of course, the same can be concluded from the Chapters 2 and 3, where already published (in scientific, peer-reviewed journals) research papers are presented. However, the introductory part (Chapter 1) gives the impression of being somehow chaotic in its content. The PhD candidate made a suitable description of relevant parts, but in those description he lost focus on the main goal of his Thesis. This led him to present many information without emphasis put on the aspect he was dealing with. This, in effect, makes the overall Introductory part too general and, moreover, lack of definitions (for example: what is the broodstock management in his opinion or if he really dealt with „broodstock management“) makes this part really confusing for the reader. This has also further implications on the perception of the following, most important Chapters (namely Chapters 2-6). Therefore, I strongly suggest that the Introductory part should be rewritten in order to provide the reader with all the important information, where the basis should be: (1) introduction of definition of how the broodstock management is considered in this study, (2) providing the information on the importance of the pond-reared broodstock in the aquaculture sector, (3) keep clarity and separation between the two main aims of fish production (i.e. keeping clear distance between the production of fish for restocking and for aquaculture purposes) for either commercial production or restocking as well as (4) keep clear message if and how the aspects of stress and mortality of pikeperch broodstock were addressed by other authors.



The Chapters 2, 3 and 5 are pretty consistent in the overall aim of those studies. The Authors focused on how to manage the broodstock (having in mind mostly the welfare and reduction of mortality) during the semi-intensive spawning operation. There was lot of effort put on these experiments aiming at understanding how to provide appropriate conditions for spawning on the nests, what are the physiological reaction and how to manage the fish after this stressful operation. And despite these three Chapters are clear enough from the perspective of the aim of the Thesis, the Chapters 4 and 6 seems to be somehow inconsistent in their meritoric content with the other parts. Chapter 4 refer to the collection of the sperm for *in vitro* fertilization, whereas argumentation of the PhD candidate was that the nest spawning of pond-reared or wild fish is much more beneficial for the farmers due to the reduction of the handling stress being direct reason of high post-spawning mortality. So, this Chapter is pretty inconsistent with the overall sound of the Chapters 2, 3 and 5. Therefore, I strongly suggest to justify the inclusion of this Chapter by informing the reader the link between the concept of „nest spawning“ developed and the collection of the sperm *in vitro*. Another confusion is related to the Chapter 6 which aims at investigation of the foraging behaviour of the fish during the wintering period in the tanks. The Authors of this study, with PhD candidate being the first author of this manuscript (the paper was not published yet) made interesting study, but there is no relation to the spawning. So, in effect the authors have made lot of work without really addressing whether the observations they made are having any implications to reproduction. Besides the males and females were investigated separately, there is no relation to the broodstock. In effect, this study is another one barely linked to the overall goal of this study – broodstock management. In effect, I strongly suggest to either exclude this Chapter from the Thesis or introduce suitable rationale for leaving it within the structures of the Thesis.

Chapters 2, 3 and 5 are already published in scientific, peer-reviewed journals, what increases the overall quality of the Thesis. In the Chapters 2 and 3 the PhD candidate is the first and corresponding Author what confirms the skills of the PhD candidate to prepare the scientific articles, properly report the scientific data and conduct meritoric debate with the other members of the scientific community – being undoubtedly the highly important attribute of the scientist. It should be highlighted that in Chapters 4 and 5 the PhD candidate was the co-author with contribution estimated to 30 and 20%, respectively. However, this should not be considered as a critics on the scientific abilities of the PhD candidate as I assume these were his first steps in research where he was working under the supervision of more experienced researchers acquiring necessary knowledge and skills.

Chapters 4 and 6 are the manuscripts of the articles indicated as „submitted“ suggesting that these articles are being currently evaluated for publication in scientific journals. And due to this reason I have made a close look on these manuscripts in order to rate the scientific content of those experiments. Chapter 4, besides its suitability for this Thesis is to be explained (as I mentioned earlier) I have one major critics over the methodical part. The Authors in this paper were verifying whether the sperm can be stripped more than once from the same fish. In this study additional variable was tested which is catheterization – being previously recognized as the technique allowing to avoid contamination of the sperm with urine, when comparing to typically used collection of the sperm with syringe. The contamination of the sperm with urine is typically verified by analysis of the osmolality of seminal plasma, with reduction below  $\sim 300 \text{ mOsm kg}^{-1}$  indicating urine contamination. In this paper, however, the Authors recorded osmolality of the seminal plasma following collection of the sperm with catheter within the range of 216-251  $\text{mOsm kg}^{-1}$ , suggesting high urine contamination. Therefore, I strongly suggest to consider before publication omitting the aspect of catheterization and address this interesting problem (and potentially trying to explain the phenomenon of low osmolality) in future research. As considering the Chapter 6 I have no major critics on the content and overall scientific relevancy, despite the relevancy of inclusion of this study in this Thesis is questionable. Especially, that removal of this Chapter would not affect the overall scientific quality of the Thesis as well as would not affect the legitimacy of awarding the PhD candidate with the doctoral degree.



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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

Olsztyn, 01.07.2019

.....  
Date and place

.....  
Name and signature

