



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 FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Imentai Aiman, M.Sc.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Tomáš Policar, Ph.D.
Title of PhD thesis: Pikeperch (<i>Sander lucioperca</i> L.) larviculture improvements using rotifers <i>Brachionus plicatilis</i>	
REVIEWER:	
Surname: Król	Institution: Department of Ichthyology and Aquaculture, Faculty of Animal Bioengineering University of Warmia and Mazury in Olsztyn, Warszawska 117A street, 10-719 Olsztyn, Poland
Name: Jarosław	E-mail: krolas@uwm.edu.pl
Titles: Assoc. Prof.	
Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: reproduction and larviculture of percid fish 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):

PhD thesis titled „Pikeperch (*Sander lucioperca* L.) larviculture improvements using rotifers *Brachionus plicatilis*“ written by M.Sc. Aiman Imentai deals within a very important issue regarding diversification of European freshwater aquaculture. Pikeperch, main object of presented research, is considered among the most promising species for possible intensive fish farming in Europe. Despite considerable progress of aquaculture production of this species observed within the last three decades, further expansion of commercial production has been still restricted due to the low possibility of control over the reproduction and high unpredictability of stocking material quality. One of the key to the further development of the pikeperch production is to optimize conditions for rearing the larval stages. Larviculture has been recognized as one of the main bottlenecks in the percid fish aquaculture, mainly because of high mortality of larvae resulting from problems with swim bladder inflation, non-feeding behaviour or intra-cohort cannibalism. Moreover, one of the most important issue for pikeperch larval husbandry optimization are first feeding by using live feed and weaning procedure improvements.

The results presented in PhD thesis of M.Sc. Aiman Imentai have been already published or submitted in peer-review, good quality journals in the field of aquaculture and fisheries science (*Aquaculture, Aquaculture International and Journal of Applied ichthyology*). M.Sc. Aiman Imentai is a first and corresponding author in 3 papers (two published and one submitted to *Aquaculture*



journal) with her contribution about 50-60% and co-author of one publication with low share about 10%. Despite the latter, in overall I consider that her participation in obtaining results of the presented research was significant and scientific importance of the thesis is high, in both aspects, for basic research (regarding knowledge of feeding behaviour of pikeperch larvae) and applied research (regarding improvement in pikeperch larviculture production).

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):

PhD thesis titled „Pikeperch (*Sander lucioperca* L.) larviculture improvements using rotifers *Brachionus plicatilis*“ written by M.Sc. Aiman Imentai on 95 pages is compounded in six chapters including: (1) general introduction with main objectives of PhD thesis and list of references (chapter 1, 7-21 pages); (2) copies of three publications and one submitted manuscript corresponding to specific objectives, methods used, obtained results and conclusions related to presented research (chapters 2-5, 22-76 pages); (3) general discussion with overall conclusions (chapter 6, 77-84 pages). Finally, summary in English and Czech, with scientific activity of the PhD student are also presented (86-85 pages). Reviewed PhD thesis is well written with high comprehensibility and logicity of the chapters as well as their ordering are correctly arranged. Selected publications and manuscript are consistent with objectives and theme of PhD thesis. Most of the results presented in the thesis were subjected to a very strict reviewed process before publishing them in the good scientific journals and thus were validated for adequate scientific level and in my opinion does not need to be further evaluated by additional reviewer.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

Optimization of management protocols in pikeperch larviculture, including standardization of the initial exogenous feeding, enhancement percent of larvae with inflated swim bladder and mitigation of intra-cohort cannibalism for production high quality larvae on commercial scale is still need. Research presented in this PhD thesis, relating to implementation standardized protocol of first feeding larvae from mariculture to pikeperch larviculture was an important scientific attempt to resolve one of above bottlenecks in percid fish culture. The presented research was planned in a well thought out and carried out in a logical and coherent manner, which resulted in a well-written doctoral dissertation. As indicated above, most of the results presented in the thesis were published in good scientific journals and does not need to be further evaluated, therefore I will limit myself to presenting the objectives of these work and the most important results/conclusions obtained therein.

Chapter 1. Introduction

The first chapter of PhD thesis concerns general information of the pikeperch aquaculture production in Europe with special emphasis to main bottlenecks of larviculture of this species and discussion of the research problem to be solve by Author of this thesis. This Chapter is well written and supported by recent bibliographic references. Objectives of presented thesis are clear formulated and resulted from scope of prepared research.

Chapter 2. Introduction of rotifers (*Brachionus plicatilis*) during pikeperch first feeding (based on



article written by Yanes-Roca et al., published in Aquaculture 497: 260-268; 2018)

The objective of presented study was to optimize survival, growth and fitness of pikeperch larvae during the first feeding by using rotifers as the first live prey instead of artemia nauplii. The results showed a significant increase in survival of pikeperch larvae fed rotifers for initial 3 days followed by feeding on Artemia and combination of rotifers and Artemia for the following 9 days. The highest SGR was found in the group fed with combination of rotifers and Artemia after 3 day rotifer feeding. However, once larvae reach a critical size, rotifers become too small. Authors recommend feeding pikeperch larvae by rotifers during the first 12 days post hatch, followed by a period of co-feeding with Artemia and gradually wean larvae off rotifers as they grow out. In conclusion, Authors pointed to the need for future research to establish optimal weaning periods using combination of rotifers/Artemia as a first feed in pikeperch larvae.

Chapter 3. Effects of first feeding regime on growth performance, survival rate and development of digestive system in pikeperch (*Sander lucioperca*) larvae (based on manuscript written by Imentai et al., submitted to Aquaculture; 2020)

The aim of presented study was optimization of the first feeding regime for pikeperch larvae using rotifers and Artemia under controlled conditions. The assessment of the nutritional status of larvae was support by observation of histological development of liver, intestine and exocrine pancreas. In conclusion, Authors stated that feeding pikeperch larvae with *B. plicatilis* from 5 to 8 DPH and afterwards exclusively with Artemia or combination of rotifers and Artemia till 17 DPH resulted high survival and growth rates, and better development of digestive organs and they recommended such protocol as an optimal feeding regime of pikeperch larvae.

Chapter 4. Optimized application of rotifers *Brachionus plicatilis* for rearing pikeperch *Sander lucioperca* L. larvae (based on article written by Imentai et al., published in Aquaculture International 27: 1137–1149; 2019)

The goals of presented study were to evaluate the effects of salinity level (0‰, 2‰, 4‰, 8‰, and 16‰) on (1) motility of rotifers *B. plicatilis* in a water column; (2) gut fullness of pikeperch larvae fed on *B. plicatilis*; (3) survival, growth, and intestine morphology of pikeperch larvae fed on *B. plicatilis* to 11 DPH.

Obtained results showed that rotifers stocked into freshwater were completely immotile and exposure of rotifers to salt water resulted in a significant dose-dependent increase in the percentage of motile *B. plicatilis*. The gut fullness of pikeperch larvae fed with rotifers was significantly lower at 8‰ salinity than in other tested groups. The highest mean survival and SGR were obtained at 2‰ and 4‰ salinity and significantly differed from 8‰ and control group at 11 DPH. Salinity of 16‰ resulted in 100% mortality in pikeperch larvae.

Chapter 5. Effect of *Brachionus plicatilis* density on pikeperch (*Sander lucioperca* L.) larva performance at first feeding (based on article written by Imentai et al., published in Journal of Applied Ichthyology 35: 1292–1294; 2019)

The aim of the last study was to determine the minimum *B. plicatilis* density as a effective for acceptable survival and growth of pikeperch larvae during first exogenous feeding.

Growth of pikeperch larvae was significantly affected by rotifers density at the onset of exogenous feeding. A high survival rate was found among all tested groups with no significant differences. In conclusion, Authors suggested using density of rotifers in level of 6 mL⁻¹ at 16.5°C as suitable for the nutritional requirements of pikeperch larvae from 5 to 9 DPH.

Jul



Chapter 6. Discussion with conclusions

Discussion part is also well written, taking account all elements one by one and discussing the main results brought by the work. Conclusions of PhD dissertation, presented at the end of this chapter, are comprehensive and based on obtained results.

During the oral defence, I would like to ask PhD candidate about her opinion relating to possible implementation of obtained results into commercial production of pikeperch on fish farms in Europe. Especially, I'm interested in the technical and economical aspects of using rotifers and brackish water in larviculture of the percid fish species.

Concluded, the PhD thesis of M.Sc. Aiman Imentai is a valuable contribution to our knowledge on pikeperch larviculture. The work represents high quality research supported by reliable results which were published in reputable scientific journals. The PhD candidate adequately demonstrated her readiness for an independent scientific work. Therefore, I recommend M.Sc. Aiman Imentai for next step in PhD defending process.

FINAL RECOMMENDATION

PhD Thesis can be recommended for defence

2.07.2020, Olsztyn

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Date and place

Jaroslav Król

Name and signature

Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Imentai Aiman, M.Sc.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Tomáš Polícar, Ph.D.
Title of PhD thesis: Pikeperch (<i>Sander lucioperca L.</i>) larviculture improvements using rotifers <i>Brachionus plicatilis</i>	
REVIEWER:	
Surname: Schulz	Institution: Institute of Animal Breeding and Husbandry, Marine Aquaculture, Christian-Albrechts- University, Kiel, Germany
Name: Carsten	E-mail: cschulz@tierzucht.uni-kiel.de
Titles: Prof.	
Please describe your professional relationship to the PhD student:	Please describe your field of expertise:

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 PhD thesis is focusing on the optimization of larviculture as most critical phase of pikeperch aquaculture. With this practical background MSc. Imentai Aiman is evaluating the utilization of *Brachionus plicatilis* in contrast to *Artemia salina* application as live feed organisms in pikeperch larviculture. Based on given experiences from first feeding of marine fish larvae, various experiments were carried out to identify optimal management of *Brachionus plicatilis* application in pikeperch larviculture. The experimental set ups were highly complex, time consuming and needed elaborated skills in fish husbandry and plancton culture. The PhD student were able to gain very promising results as mortality and growth of larvae could be optimized by newly established first feeding methods of pikeperch larvae. Beside this applied relevance, presented PhD thesis offers many basal information about intestinal development and nutritional demands of pikeperch larvae. Impact of presented thesis is therefore extraordinary in various matters.

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 presented thesis is well structured in 6 chapters with an initial introduction and final general discussion. In the chapter 2-5 empirical work is presented in form of manuscripts of already published (chapter 2,4,5) manuscripts or submitted (chapter 3). In chapter 3-5 Imentai Aiman is the first author and in chapter 2 in a line with 6 authors (at position 5).

Based on a very general introduction the Imentai Aiman is aiming to provide all necessary information to describe theoretical background of various research questions to be elaborated in the following chapters. Some of presented paragraphs (e.g. 1.2.1 Recirculating aquaculture systems; 1.4.2 Swimm bladder inflation) are not directly linked to the overall topic and resulting research tasks. Nevertheless they help to understand the broad topics range to be taken into account, when working with pikeperch. For the precise research tasks of each following chapter a bit more specific introduction would be beneficial as underlying scientific drawbacks and bottlenecks are not conclusively presented. I would appreciate, if the candidate could include this in the introduction. The order of the following chapters are logically perfectly structured as the reader recognize the growing knowledge gained within the study and obvious research questions opened and solved in follow up experiments. The linguistic quality in the published MS is precise and adequate, while in the introducing and also the general discussion chapter a more scientific expression and revised orthography would be welcome.

Nevertheless, the extraordinary experimental set ups, gained results and overall thesis structure are completely meeting the requirements for the preparation of a PhD thesis.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

As pikeperch has been realized as one of the promising candidates for aquaculture the development of efficient culture techniques are needed. Currently one of the limiting bottlenecks in pikeperch aquaculture represents first feeding of fish larvae as it is accompanied with high mortalities. Since rotifers *B. plicatilis* are established especially in marine fish larvae cultures, they are not been used for pikeperch larval culture yet. Therefore many questions regarding first feeding of pikeperch larvae with rotifers are focused in the presented PhD thesis.

The effects of *B. plicatilis* on survival rate, growth performance and fitness of pikeperch larvae during first feeding were evaluated in the first study (Chapter 2). Larvae were reared under three different diets (Artemia; Artemia/rotifers; rotifers) from 3 till 17 days post hatch (DPH). Using rotifers as first diet for pikeperch larvae was shown to benefit survival and growth rates. Larvae fed on rotifers only or combined diet obtained higher survival and growth compared to Artemia diet. It was found that dietary essential fatty acids influenced

fish larvae fatty acid profile, although the low EPA and high DHA content of rotifers fed pikeperch larvae in contrast to the fatty acid composition of other treatments were highly surprising. Based on the given results authors concluded that the mixed diet of Artemia and rotifers could be recommended as first feeding in pikeperch larviculture. The initial manuscript published in the journal Aquaculture is a solid piece of work with a specific applied focus. The results are relevant and presented in a proper way, although figure 4 could be a bit more simplified to be easily understood. In addition the surprising fatty acid profile of rotifers fed pikeperch larvae could be more highlighted and discussed on possible metabolic pathways. Nevertheless, the presented MS serves as base for various new research issues in the follow up experiments.

The objective of the second experiment, presented as manuscript to be (or already) submitted, was to optimize the first exogenous feeding regime for pikeperch larvae using rotifers and Artemia to specify the results of previous study. Larvae were therefore fed with rotifers for 3 days and afterwards from 8 to 17 DPH they were adopted to 5 different regimes as follows: A) only rotifers; B) 8-13 DPH rotifers/14-17 DPH Artemia; C) 8-10 DPH rotifers/ 11-17 DPH Artemia; D) only Artemia; E) a combination of rotifers and Artemia. It was found that feeding pikeperch with rotifers from 5 to 8 DPH and afterwards exclusively with Artemia or with rotifers and Artemia mix till 17 DPH can ensure high survival and growth rates, and rapid development of digestive organs. Feeding larvae with rotifers from 5 to 8 DPH and afterwards replacing with Artemia till 17 DPH is recommended by the authors as an optimum feeding regime, although fish fed treatment C or E were on a comparable level. In addition it remains unclear, why survival of group c were statistically different to D or E as mean and variation were on an obviously comparable level. Furthermore, it would be helpful to include the coefficient of growth variation as factor as it probably influences cannibalism and survival in pikeperch larviculture.

In the following third study published in Aquaculture International it was aimed to determine the optimal salinity for rearing of pikeperch larvae using *B. plicatilis*. Rotifers were stocked under different salinities (0‰, 2‰, 4‰, 8‰, 16‰) and motility was investigated over a 6-h period. The same salinities were used in second trial of this study to quantify the effect on pikeperch gut fullness over the course of 11 h. In the third trial, the survival and growth rate of larvae from 4 to 11 DPH at low and medium salinities were analyzed. Results of this study showed that rotifers stocked at all tested salinities, except for freshwater retained motility for more than 6 h. Gut fullness increased after each feeding in 2 and 4 ‰ as well as in the control group although informations in Fig 2 are somehow complicated to extract. Pikeperch larvae reared at 2‰ and 4 ‰ showed higher survival and growth rate during trial compared to freshwater and 8 ‰ treatment. The authors concluded that larvae rearing in low salinity water had higher survival and growth rate during initial exogenous feeding with *B. Plicatilis* although treatment without salt supplementation showed even lower survival rates.

In the final study (Chapter 5) Aiman Imentai aimed to determine the optimal rotifers density for pikeperch larvae at the beginning of exogenous feeding. The study is presented as short communication published in the Journal of Applied Ichthyology. Larvae were divided into 4 groups at 87 different rotifers densities (2 ind/mL, 6 ind/mL, 10 ind/mL and 20 ind/mL) from 5 to 9 DPH. The best growth performance was achieved at the highest rotifers density,

however survival rate did not significantly differ among the groups. The results suggested that higher *B. plicatilis* densities can be considered optimal for larval growth from 5 to 9 DPH. In the final general discussion chapter Aiman Imentai is discussing her main points with results available in the scientific literature in a straight way. In addition she provides clear conclusions based on given results.

Overall assessment:

The thesis represents a comprehensive examination of the first feeding of pikeperch larvae with live feed. In addition to being mostly well written, the four thesis chapters are appropriately detailed with respect to methodology and have been defensibly conducted with respect to statistical analyses.

I very much appreciated the clear structure and thematical development within the thesis. Furthermore, I discovered in the specific chapter a solid evidence of critical thinking by the candidate, particularly in areas in which she clearly expressed an ability to think beyond the intellectual and methodological boundaries of her thesis projects. All chapters are already published or are most certainly publishable (chapter 3) and they represent the culmination of extensive, broad, and quite demanding work on the part of the candidate. This is a highly commendable characteristic that will serve the candidate well. My critics regarding the very general introduction and specific issues in chapter 3 are of minor relevance in contrast to the importance of gained results. Nevertheless, I appreciate if the PhD candidate could check these issues before final printing

In sum, I recommend acceptance of the dissertation for defence without any reservations.

FINAL RECOMMENDATION

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

Kiel, 22.06.2020
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