



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Jiri Kristan, Dipl.-Ing.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Tomáš Polícar, Ph.D.
Title of PhD thesis: Optimization of reproduction and gamete quality in percid fish	
REVIEWER:	
Surname: Kucharczyk	Institution: University Warmia and Mazury Oczapowskiego 2 str. 10-719 Olsztyn Poland
Name: Dariusz	
Titles: Prof.	E-mail: darekk@uwm.edu.pl
Please describe your professional relationship to the PhD student: I know the PhD student and I know his published papers. He visits my laboratories in 2012. He had some small cooperation with one of the PhD student from my department.	Please describe your field of expertise: I'm working on improving artificial reproduction of fish under controlled conditions, mainly wild cyprinids. But one of the main topic in which I work is the artificial reproduction of percid with special emphasis on the quality of gametes and larvae. The obtained results in these studies are implemented in fish farms and used in commercial production.

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 doctoral dissertation by Jiri Kristan is a part of the modern research on the improvement of reproductive biotechnology and rearing larvae of fish which have potential interest to intensive aquaculture. In recent years, the European aquaculture is looking for new species to extension of culture, both freshwater and marine. Among freshwater fish, one of the most valuable species are percids: Eurasian perch and pikeperch. The production of these species is developed year to year. However, the preparation of the full protocol of culture requires, on the one hand the aspects of basic



research and on the other, the applied research. The doctoral dissertation by Jiri Kristan entitled “Optimization of reproduction and gamete quality in percid fish” research fits precisely in this current trend of research. They are competing to research carried out by other authors in Europe. There are also carried out on a very high scientific level. The compilation of the results obtained in different European research teams should made fast developing of intensive percid aquaculture in Europe.

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 PhD thesis by Jiri Kristan entitled “Optimization of reproduction and gamete quality in percid fish” is correctly structured. The aim of the study was clear and understandable presented. The chapters are ordered directly: first the chapter about the broodstock, later the chapters containing chosen aspects of artificial reproduction, including application of anesthetic solutions, and later investigations of gametes (spermatozoa) quality and methods of elimination pikeperch eggs stickiness. The author chooses the adequate and responsible research methods to solve the PhD study objectives. The quality of published and submitted papers to the scientific journals (chapters 2, 3, 4, 5, 6 and 7) are high. All attached papers and manuscripts are ordered logically. The aim of the study is strictly correspond with the described results. The PhD thesis contains important information for scientists and fish farmers. Much of the information contained in this trial can be successfully implemented immediately to commercial fish culture.



OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The doctoral dissertation by Jiri Kristan entitled “Optimization of reproduction and gamete quality in percid fish” is a part of the modern research on the improvement of reproductive biotechnology and rearing larvae of fish which have potential interest to intensive aquaculture. The research was done on two freshwater fish species, which has commercial value today, but in the nearest future Eurasian perch, *Perca fluviatilis* and pikeperch, *Sander lucioperca*, will be very important aquaculture species. The commercial culture on the mass scale need very well working protocol of:

- ✓ Propagation, culturing and handling with the spawners.
- ✓ Technology of artificial reproduction, both in season and in out-of-season, including obtaining high quality gametes.
- ✓ Methods of hatchery techniques including eggs incubation.
- ✓ Larviculture.
- ✓ Culture of juveniles and adult fishes to the market size.

The bottleneck of whole production process is artificial reproduction. This part of culture is ending in the case of obtaining larvae. Without developing of full protocol of artificial reproduction of Eurasian perch and pikeperch, the mass culture In the PhD thesis Jiri Kristan focused on few very important problems which solving increasing success of percid culture. The procedures on handling spawners and use anesthetics during manipulation are very important, because the Eurasian perch and pikeperch are very sensitive for stress. Stressed fish produced low quality gametes and showed high mortality rate. Some new, but very important information from these aspects are present in the PhD thesis. The improvement methods of artificial reproduction, including applying new hormonal spawning agents, testing the doses of these agents and hatchery techniques gives the possibility to fast developing methods of mass aquaculture of percids in Europe. The PhD thesis has identified few possible problems in percid reproduction, and presented and discussed factors having the potential to improve embryo production for pond or intensive percid aquaculture.



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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

Olsztyn, 2013-06-16
Date and place

...Dariusz Kucharczyk
Name and signature



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Title of PhD thesis: Optimization of reproduction and gamete quality in percid fish	
REVIEWER:	
Surname: Fontaine	Institution: University of Lorraine 24-30 rue lionnois Nancy 54 003 France
Name: Pascal	E-mail: p.fontaine@univ-lorraine.fr
Titles: Pr	
Please describe your professional relationship to the PhD student: No link with the PhD thesis	Please describe your field of expertise: Aquaculture, fish domestication, fish reproduction, regulation of reproductive performances

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**):

Percid fish culture (Eurasian perch, pikeperch) is one of the major ways identified in Europe to promote a diversification of the inland aquaculture. More than 20 farms have been built in Europe over last 10 years using mainly intensive RAS (Recirculated Aquaculture Systems) technology. They produce now around 1000 tons per year. To sustain the development of this new percid fish culture sector, it is necessary to secure the performances of the newly-built hatcheries. They require the development of reliable protocols for the reproduction control of both species, including broodstock anaesthesia before handling, hormonal treatments for artificial propagation, control of sperm quality, elimination of egg stickiness ... Considering this framework, this PhD thesis develops an applied research focused on major specific and targeted bottlenecks and supplies original data which will be very useful for percid farmers.

This PhD thesis constitutes a new and real scientific input for optimization and standardization of several protocols useful for improving broodstock management during spawning season.



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 PhD thesis is based on a compilation of six independent studies published (including two only submitted) under different forms (5 articles in peer-reviewed journal + 1 practical handbook). That means that the candidate had as a strong objective the publication of its original data obtained from his experimental studies. The main common link between all these studies is the improvement of Eurasian perch and pikeperch broodstock management for culture purposes.

However a limit of such approach is that a specific scientific question is not thoroughly studied and consequently the general discussion (4 pages) is very limited. This part appears rather as an extended abstract than a real general discussion.

Here I must underline that it is a „French point of view“ which doesn't know really what is recommended or acceptable for a Czech PhD thesis!

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The PhD manuscript deals with the „Optimization of reproduction and gamete quality in percid fish“ (116 pages). It is compounded by 8 chapters : an initial introduction, two chapters on Eurasian perch reproductive biology, four chapters on pikeperch reproductive biology and a last chapter for a general discussion.

The introduction (first chapter, 4 pages) give us a concise state of the art concerning firstly the interests of percid fish for human consumption and related markets and secondly the main current bottlenecks related to percid reproductive biology.

The second chapter concerns an experimental study focused on the comparison of reproduction characteristics and broodstock mortality in farmed and wild Eurasian perch (*Perca fluviatilis*) females during spawning season under controlled conditions. It has been published in Turkish Journal of Fisheries and Aquatic Sciences (IF : 0.432, year 2012). Based on the analysis of broodstock morpho-anatomical parameters and mortality, female reproductive activity, fertilization and hatching rates, and larvae quality, significant effects of domestication level (farmed vs wild) are observed on female absolute and relative fecundity, and hatching rate. However the initial weight and length of both broodstock are significantly different, whereas such broodstock characteristics are correlated to female fecundity. This point should be more discussed.

The third chapter concerned „the comparison of the effects of four anaesthetics on haematological and blood biochemical profiles in pikeperch (*Sander lucioperca* L.). It has been published in Neuroendocrinology Letters (IF : 1.296) in 2012. The study shows that clove oil must be



recommended for pikeperch manipulation (lowest effects) whereas propiscin and 2-phenoxyethanol are not suitable for pikeperch manipulation.

The fourth chapter is long (21 pages) and focused on „artificial and semi-artificial spawning in Eurasian perch (*Perca fluviatilis* L.) for mass embryo production. In fact, this chapter is a very applied research (with few original data) comparing the interest of different technics for mass larvae production in farm conditions. In my opinion, this chapter is too applied for a PhD thesis.

The fifth chapter is entitled „hormonal induction of ovulation in pikeperch (*Sander lucioperca*) using human chorionic gonadotropin (hCG) and mammalian GnRH analogue“. This study is in press and will be published in the review *Aquaculture International* (IF : 0.912) in 2013. This study has identified successful protocols (100 % of ovulation rate, latency time 78-83 hours) using hormonal intramuscular injection (based on a single injection) with either hCG (500 or 750 IU.kg⁻¹) or a mGnRHa (Supergestran, 25 µg. kg⁻¹). Higher hatching rates were observed with hCG treatments (85 % vs 60 %).

The sixth chapter concerns „sperm morphology, fine structure and motility in pikeperch, *Sander lucioperca* (Percidae, Teleostei) using different activation media“. This study has been submitted for publication to the review *Czech Journal of Animal Science* (IF : 1.079). It is shown that pikeperch and Eurasian perch sperms have similar morphology and fine structure, in spite of differences observed in arrangement of midpiece and centrioles and presence of fine along the flagellum. The use of an activation medium containing Ca²⁺ for sperm activation of short-term stored sperm is suggested.

The seventh chapter deals with „Alcalase treatment for elimination of stickiness in pikeperch (*Sander lucioperca* L.) eggs“. This study has been submitted for publication to the review *Aquaculture* (IF : 2.041). The application of alcalase enzyme (1.5-2 mL.L⁻¹) can be successfully used for elimination of pikeperch egg stickiness, with less time than traditional milk/clay/talc methods.

Finally, a short general discussion (4 pages) ends the PhD manuscript and summarizes all major results obtained in the successive experimental studies. However the perspectives from this PhD thesis should be more discussed.

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

June 2013, 12th at Nancy (France)

Date and place

Pr Pascal FONTAINE

Name and signature