Fakulta rybářství
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: Viktor W. Švinger, Ing.	**First name(s), surname, titles of supervisor:** Prof. Dipl.- Ing. Jan Kouřil, Ph.D.
Title of PhD thesis: Optimization of Hormone-Induced Ovulation in Economically Important Fish Species	

REVIEWER:

Surname: Wedekind	**Institution**: Institut für Fischerei Weilheimer Str. 8 82319 Starnberg Germany
Name: Helmut	
Titles: Dr. sc. agr.	**E-mail**: Helmut.Wedekind@lfl.bayern.de
Please describe your professional relationship to the PhD student: none	**Please describe your field of expertise:** aquaculture, fishfarming, research/education

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 study is dealing with a very actual scientific topic. Even if there is a good basic knowledge on endocrinology and mechanisms of reproduction in fish, there is still a lot of information on the transfer of scientific understanding to aquaculture practice needed. Therefore, the study is very important for the significant field of fish reproduction in aquaculture. The dissertation-project represents a sythesis of scientific and practical farming approaches. The experimental studies included are of high scientific value and were very much orientated on the application in the field of fish production. In the international context the work is quite competitive. There are not many studies combining basic and applied research with practical aspects, as can be found here.

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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, logicality 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)**:

In a very extensive introductory chapter 1 a qualified overview was given on the scientific state of the art of the physiological mechanisms of hormone treatments in fishes. Besides historic data, actual publications were analyzed accurately. The cited publications are of high significance for the project, and their findings were accurately included in the own experimental work.

The following chapters 2 – 5 contain different experimental approaches on the optimization of hormone induced ovulation in different fish species. Every paper included is based on a suitable literature-overview, followed by interesting experimental investigations. The results were accurately discussed and interpreted. The publications represent relevant approaches in this field of research, and are as such a proof for the high academic level of the accomplished research. The investigations were presented in the thesis in a logical order. Altogether, the thesis' main topic was investigated with adequate methods and quite original scientific designs. As a result, new findings on hormonal treatments as an effective tool to advance and synchronize ovulation in brook char, whitefish, grayling, and brook trout were worked out. The description and interpretation of the research is very accurate.

The publications included in the thesis demonstrate the high level of the work done by the candidate. Their results were adequately analyzed and interpreted in the final discussion and led to new scientific and knowledge.

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OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The thesis titled "Optimization of Hormone-Induced Ovulation in Economically Important Fish Species" by Victor W. Švinger is dealing with a very actual topic, which is not only of scientific relevance, but also for practical fishfarming. As aquaculture is developing dramatically all over the world, the control of reproduction in cultured fish species is of major importance. Controlled reproduction and propagation of fishes is a basic precondition for future improvement and growth in this sector.

In the thesis, the author uses a very systematic methodology:

Historical and recent publications on hormonal treatment of a wide range of fish species were analyzed and summarized. For this, especially basic literature on fish physiology was investigated. The author succeeded to work out analogue interpretations and in the application of historic experiences and methodology to his own studies. It is very positive, that in the following own experiments actual aquaculture practices were referred at. As such, the literature study is very extensively, it was carried out in an accurate and systematic way. As a result, a complete summary of the hormone treatments for induction and synchronization of ovulation in salmonids existing in the scientific literature of the last decades was given. Out of this, the major aims if the dissertation-project were worked out clearly – quite understandable for the reader.

It was worked out, that there is an urgent need for scientific research in the field of

synchronization and artificial reproduction in important aquaculture species, especially relevant under the conditions of the Czech Republic. Moreover, the project is of general importance due to the worldwide relevance of this subject for other species and farming systems.

As the first aim of the dissertation the brain-pituitary-gonad axis in salmonids was identified. Together with the focus on regulatory factors and mechanisms on the reproductive cycle, e.g. photoperiod, temperature, and their interactions with the endocrine system in fishes, the regarding experimental research was carried out on a sophisticated level. This can be followed up in the impressing first paper (Chapter 2), published in an international scientific journal. Here the successful application of different hormone treatments of brook trout was published. It was impressively demonstrated how synchronization of ovulation can be reached by synthetic GnRHa preparations. This publication represents a real improvement of knowledge about the propagation of salmonid species and gave novel information on the reproductive physiology in fishes.

In the second paper included in the thesis (Chapter 3) it was shown, that hormonal treatment is an effective tool to advance and synchronize ovulation in whitefish. This research gave fairly new knowledge on the reproduction of whitefish, a group of species with a significant potential in aquaculture. The research gave new and quite relevant findings on the particular species (*C. peled*) and improves general knowledge on synchronization and its effect on gonad quality.

In Chapter 4 new aspects of another species, *Thymallus arcticus arcticus*, were investigated. The paper is the first detailed report on controlled reproduction induced by hormones in this species. The findings are of basic importance and show the high level of the research carried out for this dissertation project.

Furthermore, more important salmonid aquaculture species (rainbow trout and brook trout) were focused at in Chapter 5 (submitted paper). It is dealing with the use on gonadotropic

hormones in salmonid reproduction. In this context, the focus on egg and larvae quality and the possible reasons for deformities seems to be very innovative and relevant. In detailed protocols quality assessment of gonadal products were carried out, which is a very original approach of high scientific and practical importance. This research resulted in a lot of new findings on the effect of different treatments on egg ripening and quality during artificial reproduction in salmonids.

The investigation of literature and the own experimental work are summarized and interpreted in a general discussion (chapter 6), which can be characterized as a high-level discussion. Here, the author succeeded to connect basic knowledge on reproductive biology of fishes from the literature with his own research. General conclusions on the applicability of synthetic hormones on salmonid species were made and, finally, adequately brought in touch to aquaculture conditions and practices.

In summary, the thesis is very well done. The work is a good synthesis between modern and scientific methods and practical aspects of fishfarming. The experimental approaches are modern and original. The work gave a wide range of new results, which are highly relevant for fish-reproduction under farming conditions.

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**

29.06.2013, Starnberg
Date and place

Name and signature

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REVIEWER:

Surname: Příhoda	**Institution**: Eventfish Žilina B. S. Timravy 948/6 010 08 Žilina Slovensko
Name: Juraj	
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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)**:

In general, the submitted dissertation represents original work, from theoretical point of view is correct, and deals with highly topical issue – hormonal stimulation of reproduction process in salmonids. Modern methodical procedures were used, while a number of new theoretical as well as practical findings have been presented. Those are applicable not merely in the Czech republic but also in other countries. By this dissertation, the author has proved to be able of creative scientific work and showed abilities to put his deep theoretical knowledge into practice.
By overall impression of the thesis I would like to note that its quality level meets the solicitude of the author´s efforts he has put into, and that also his foreign experiences gained during foreign internships are expressed herein as well as the solicitude of the supervisor, Prof. Dipl.-Ing. Jan Kouřil, Ph.D., and other co-workers. By its quality level, the dissertation is comparable with current most modern standards of similar dissertations at international level and therefore able to compete.

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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, logicality 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 dissertation contains 117 pages and is divided to 6 main chapters. Introduction refers to very elaborated 170 citations which are incorporated in 8 subchapters containing current knowledge from area of hormonal stimulation of ovulation in fish, the need and options of its utilization, and the history of hormonal stimulation in salmonids. Third chapter includes author´s primary objectives of the dissertation:

- create and improve procedures of stimulation of ovulation using synthetic GnRHas in economically important and endangered salmonid species
- first verification of GnRHa application in brook trout (*Salvelinus fontinalis*), Northern Whitefish (*Coregonus peled*), and Arctic grayling (*Thymallus arcticus*)
- optimize dose and delivery of GnRHa
- post-application survival of the broodstock
- impacts of GnRHa on egg survival and egg quality

Other chapters describe in detail the natural mechanism of hormonal stimulation, their control factors such as photoperiod, temperature, use of synthetic GnRHas, delivery methods and knowledge about the effects of their application on the sex product quality. Very important is the summarization of the literature resources into an informative table showing used hormone preparations in particular salmonid species, dosages, and also authors of course. Next chapters (2□5) contain authors individual works:

- Synchronization of ovulation in brook char (*Salvelinus fontinalis*, Mitchill 1814) using emulsified D-Arg^{6}Pro^{9}NEt -sGnRHa
- Synchronization of ovulation in cultured northern whitefish (*Coregonus peled*, Gmelin 1788) using (D-Arg^{6}Pro^{9}NEt)-sGnRH analogue and its effect on egg quality
- Induction and advancement of ovulation in wild Arctic grayling (*Thymallus arcticus arcticus*) using D-Arg^{6}Pro^{9}NEt -mGnRHa Lecirelin
- Effects of D-Arg^{6}Pro^{9}NEt -GnRHa on reproductive success and egg size in rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*).

The papers have a classic structure of introduction, material and methods, results, discussion and conclusive remarks supplemented with transparent tables and figures featuring regression and correlation curves.

All papers have already been published or are currently in press in scientific journals (Aquaculture International, Aquaculture Research, Czech Journal of Animal Science).

The Chapter 6 contains general discussion about the problematic of usage of synthetic gonadotropin releasing hormones according to authors own experience supplemented with literary references, further English and Czech summary, thanks, current author´s publications, study plan and CV.

Please write comments in extent of 1-2 pages:

The ray-finned fish of the order *Salmoniformes* are characterized by missing or rudimentary oviduct. After the follicle and ovarian rupture, ripe eggs are released into the body cavity, from which are laid outside of the fish through urogenital papilla. Current salmonids arose from three lineages: whitefish (*Coregoninae*), graylings (*Thymallinae*), and the char, trout and salmons (Salmonidae). From these lineages or families came experimental fish in this dissertation. Conditions described by the author in the Introduction strongly affect not merely the timing of ovulation but also the sex product quality and alevins quality mainly during endogenous development. Most examined are these conditions in rainbow trout (*Oncorhynchus mykiss*) being currently the most farmed salmonid for commercial purposes. Therefore mainly through selection, photoperiodic and water temperature manipulation, the eggs can be harvested the all year round. However, in wild salmonid fish and species, although commercially reared, the reproductive cycle shows non-synchronized shape and spawning season exceeds a very long period. This necessitates intensive handling and long-term starving in a measure of weeks, which negatively affect health and often results in fungal disease and increased mortality.

The most problematic is capture of the wild broodstock from free waters. In case of badly timed capture (capture has to be performed either during spawning migration or directly on the spawning grounds) or sharp fall of temperatures, the artificial reproduction frequently ends with negative result. Hypophysation procedures (used mainly in carp reproduction) fell by the wayside in salmonids.

Therefore the objectives of this dissertation regarded optimization of hormone controlled reproduction using GnRHa in brook trout, Northern whitefish and Arctic grayling. Because it was a first use of GnRHa in these species, optimization of dosage, GnRHa delivery and estimation of effects of theses procedures on the egg quality (morphological characteristics and viability) and post-spawning broodstock survival had to be carried out. Similarly to other salmonids, it has been found that application of GnRHa effectively synchronizes the onset of ovulation in brook trout and Northern whitefish. This is primarily important for hatchery management, because it enables accurate timing of stripping. In both species, the most effective treatment appeared to be an acute double injection of 25 $\mu g.kg^{-1}$ **D**-Arg^{6},Pro^{9}-NHEt-sGnRHa administered 2-3 days apart or injection of 25 $\mu g.kg^{-1}$ **D**-Arg^{6},Pro^{9}-NHEt-sGnRHa emulsified in Freund´s incomplete adjuvant, which ensures sustained release of analogue with much more effective stimulation of LH secretion. This procedure does not require repeated injections. From practical point of view is this method very important since it reduces handling. Similarly very important finding is that double acute injection of **D**-Tle^{6},Pro^{9}-NHEt-mGnRHa (Supergestran®) at 10 µg.kg-1 effectively induced ovulation in Arctic grayling 1□1,5 month ahead of natural spawning time, which enabled quick stripping and release of broodstock back to the natural water. In the figurative sense, this would enable also capture of European grayling early in the pre-spawning season during good weather conditions, because particularly bad weather often hampers capture of wild European grayling broodstock during spawning migrations. Likewise the artificial reproduction of grayling is problematic when keeping broodstock in flow-through ponds, since sudden fall of water temperature results in inhibition of ovulation and subsequent stripping. The author´s report about ineffectiveness of single acute injection is also very important.

It is of big importance, that no negative effect of GnRHa or GnRHa-FIA has been found in post-spawning period in any case. Although significant, lower viability (5-20%) of eggs coming from treated females is from practical point of view not eminent to restrict the use of these hormones in practice. It can be due to lower developmental stage of the oocyte or alternatively due to lower opening of the micropyle or deterioration of the embryonic development. Lower egg size and yolk sac fry suggest probably the lower developmental stage of the oocyte. Nevertheless, lower egg size is not the cause of the lower egg survival. Achieved results in this dissertation significantly push forward the knowledge about synchronization of ovulation in salmonids (or in Salmoniformes in general to satisfy the taxonomists, too).

It has to be noted that applied hormone preparations are registered to the fullest extent of law

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about medicaments and therefore are allowed to be used in aquaculture. I have to point out that to the extent of law Nr. 166/1999 statute book (veterinary law) injective procedures should be carried out just by the veterinarian or by a person authorized by the veterinarian in his presence.
I do not have any grave remarks to the dissertation. Just better arrangement and illustration could be devoted to the graphic projection of the action of particular hormones on the reproductive process in Chapter 1.
In general view, I would like to note that the work fully meets all criteria for drawing up a dissertation by its content, it includes all specific formalities and fulfils requirements for awarding of the scientific title Ph.D.

OVERALL COMMENTARY ON THE PhD THESIS

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**~~

Žilina, 10.6.2013
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Date and place

MVDr. Juraj Příhoda, CSc.
.....
Name and signature