



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

Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: <i>Mujahid Ali Shah</i>	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Martin Pšenička, Ph.D.
Title of PhD thesis: Sturgeon (Acipenser) represents the evolutionary transition from the holoblastic to meroblastic cleavage pattern and unique gut development.	

REVIEWER:

Surname: Yamaha	Institution: - Field Science Center for Northern Biosphere, Hokkaido University, Japan
Name: Etsuro	
Titles: prof.	E-mail: eyamaha@fsc.hokudai.ac.jp
Please describe your professional relationship to the PhD student:	Please describe your field of expertise: Embryology and developmental technology in teleost.



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**):

Historically, embryology has deepened by two scientific wheels: analysis of individual development (ontogeny) and comparison of development between species (phylogeny). In the former, developmental mechanisms has been disclosed by new technology in the area of experimental manipulation (transplantation, injection *etc.*), and genetic analysis (mutant, gene expression *etc.*). In the latter, evolutionary mechanisms have been clarified by comparison of development and/or homologous genes, such as orthologs. In the doctoral thesis by Mujahid Ali Shah, his research scheme was planned from the viewpoints of these wheels. New experimental technology was established for analyzing developmental mechanism in sturgeon. Phylogenetic process was explained in cleavage and endoderm differentiation during evolution by analysis of histological experiments and gene expression using ortholog. His plan was successfully achieved. His perspective on problem solving was creative and derived a clear answer to the object. I am convinced that the peripheral technology related to the embryonic manipulation that he has built will contribute to the life science related to aquaculture in Acipenseriformes in the future.

Last but not least, I think the university system is great because it can always provide this difficult experimental material.

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**):

- 1) Chapter connections and structuring of the main text are continuous, consistent and easy to read.
- 2) Technical developments necessary for research were newly performed, and showed potential for use.
- 3) The established techniques actually contribute to the analysis of sturgeon's development. It has been shown that the lineage of vegetal blastomeres is contribute to the nutrition for larval development, but not the establishment of three germ layers.
- 4) The embryonic development of sturgeon's endoderm cells and distribution of their descendants were clearly disclosed and compared to those of other tetrapod species phylogenetically.
- 5) The additional data in the journals of *Aquaculture* and *Fisheries Science* should be included in the manuscript of the doctoral dissertation.



OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

Comment in the introduction chapter

I was concerned about the following points,

- 1) Page 8, Fig. 1: Appearance of sexually mature adults is not *Xenopus*.
- 2) Page 9, Fig. 2: 30 min past fertilization -> post fertilization. No pectoral fin was illustrated at 48 hr.
- 3) Page 10, Fig. 3: Parentheses are not closed in *Acipenseriformes*. Reptiles and birds -> Reptiles and birds. Tetraods -> Tetrapods
- 4) Page 16, Fig. 4, and page 21, Fig. 5: Appearances of Amphibian (*Xenopus laevis*) are different from the actual ones.
- 5) Page 22, 2nd sentence, lines 3 and 5, 33, and references: Siegfried W de L, -> de Laar SW.?

Comment in the 2nd chapter.

- 1) In DD treatment, why is it that immersion in a 0.001% solution affects subsequent development, but injection into an embryo does not affect development? Is it because visible light does not enter the injected cells?
- 2) Fig. 5: Only approximate curve graph is shown. The average value, dispersion, and number of specimens are not shown in this figure.
- 3) Fig. 8: It is difficult to understand because there is no description of when the photo was taken.

Comment in the 3rd chapter.

- 1) The viewpoint of changing holoblastic eggs to the meroblastic ones is interesting, although the similar experiment had been reported in *E. coqui* previously.
- 2) I think the results of Figure 7C are interesting. This phenomenon is mentioned in the caption of the Fig. 7, but not in the text. I think that the explanation in the text is insufficient and not fully considered. Enlargement of nuclei after inhibition of vegetal blastomeres in sturgeon is similar to larger size of the nuclei in YSL than those of deep layer cells in zebrafish. It would be interesting to consider why nuclear enlargement occurs by referring to the results of experiments such as Hara and Merton (2015; Developmental Cell 33: 562-575).
- 3) In modern biology, it is often required to show not only pure biological results but also future-oriented applied perspectives. I am dissatisfied with the fact that the applied perspective is not discussed even though it is posted in an applied magazine, such as *Aquaculture*. What about the possibility of adding additional nutritional components to VP cells? Rapid growth may be expected after this treatment. This character will be required in sturgeons that take a long time to grow. Moreover, sterile individuals without primordial germ cells are useful for surrogate host of sturgeon species. In this species, which are mostly endangered species, a fast-growing surrogate host may be required in the future. I think it is necessary to incorporate an applied science perspective in your text.



Comment in the 4th chapter.

- 1) Endodermal precursor cells in zebrafish migrate on the yolk cell at the gastrula stage. In Fig. 4, do endodermal cells labelled with FITC proliferate to widen the distribution area in the ventral direction? Do they migrate on the inner surface of the lateral plate or on the yolk cells toward ventral direction? I would like a detailed description of the status of endodermal cell migration.
- 2) Fig. 6, photograph at pharyngula in Gar: only this section is a cross section at the otic vesicle region, compared with other species at this stage. It is better to show the section cut at the same antero-posterior region.

Comment in the general discussion

- 1) The author concludes that there are few maternal factors, except for germ-cell determinants, involved in morphogenesis around vegetal pole of fertilized egg. In *Xenopus*, the cortical rotation during the first cell cycle is involved in the formation of the dorsoventral axis. In sturgeon, the movement of pigment toward animal pole is observed after fertilization. Therefore, is there any possibility that the distribution of maternal molecules, which were determined in the equatorial region by authors, located around vegetal region just after fertilization and then redistributed around the equatorial region around early cleavage stages?
- 2) The author states the importance of research on jawless fishes such as lamprey. I think that the search for related genes, concerned with the accumulation of a large amount of yolk, is required. I would like to ask the author's plan as to what kind of research is possible. The reviewer has tried to manipulate the lamprey egg, but the embryo is too soft to manipulate and to microinject, because the yolk overflows from the hole after injection.
- 3) Page 93, first line and references in this chapter: Siegfried W de L, -> de Laar SW.
- 4) Page 96, third paragraph, line 1; surgeon's -> sturgeon's

FINAL RECOMMENDATION



PhD thesis can be recommended for defence



PhD thesis can be recommended for defence with reservations



PhD thesis cannot be recommended for defence

17th July 2022

Nanae Kameda, HOKKAIDO

Date and place

Etsumi YAMAHARA

山根 恒 啓

Name and signature