

Gdynia, 13.01.2026

Konrad Ocalewicz
Department of Marine Biology and Biotechnology
Faculty of Oceanography and Geography,
University of Gdańsk

Review of the doctoral dissertation by Rosella Debernardis, entitled: **The transcriptome of newly hatched larvae as a window into the exploration of parental legacy and early life performance in Eurasian perch**. The thesis was prepared at the Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn under supervision of Daniel Źarski (D.Sc.).

In the reviewed dissertation, the doctoral candidate Rosella Debernardis addresses issues linked to the larval performance of the Eurasian perch, species considered as candidate for domestication and aquaculture. Traits that are crucial for successful breeding and rearing of perch (in terms of biology and economy) including egg quality, survival, growth, proper body development and performance have been analysed in the perch at the larval stage that in the aquaculture is considered as a critical bottleneck. Moreover, transcriptome profiling, bioinformatics and statistic tools were employed to find links between mentioned above traits and transcription of maternal and paternal genes. And the mission has been successfully accomplished. Described in the thesis associations between gene transcription patterns and quantitative traits of the perch larvae enabled to receive answers for at least some of the questions Rosella Debernardis asked in the thesis what taking into account how complex and large is the dissertation I recognize as a success.

Merits-based assessment of a PhD thesis

The doctoral dissertation under review follows a hybrid format and was prepared within the framework of the doctoral school, in accordance with the guidelines set out in the Regulations for the procedure of awarding the doctoral degree at the Interdisciplinary Doctoral School of Agricultural Sciences. Its main part consists of elements typical of a monograph, but it also includes four studies detailing independent experiments conducted to verify the proposed scientific hypotheses. These studies are presented as scientific articles, one of which has been already published. The chapters containing the results of subsequent experiments are preceded by a synthetic introduction (Chapter 1) that provides a sound justification for both the research objectives and the scientific hypotheses (Chapter 2). These hypotheses were verified by the doctoral candidate through results obtained during successive experimental stages, ranging from fish rearing and the analysis of performance traits to the molecular studies. In the Introduction chapter, the candidate focuses on presenting the studied species, its role in the natural environment, in commercial fisheries, and aquaculture. She has described the species as having significant potential for aquaculture production, particularly in Recirculating Aquaculture Systems (RAS). In this part of the dissertation, the candidate outlines the life cycle of the Eurasian perch, focusing on the larval stage, which is critical for the controlled-environment farming. Perch at the larval stage present critical bottleneck due to their high mortality rates, cannibalism, interindividual variation in the growth and body malformation that are affected by environmental factors, nutrition, as well as genetic and parental effects. Here, for the first time in the thesis, the concept of larval quality (a potential of larvae for survival and proper development) is

provided. Two stages during the larval period are key for the further fate of the fish – switching from endogenous to exogenous feeding and inflation of swim bladder. PhD student has also presented how early development of fish is controlled, first by the maternal RNAs and later after activation of the zygotic genome by the parental genetic material. Maternal and paternal effects on the fish development are discussed in the genetic and phenotypic context. Issues related to larval assessments, zootechnical traits, role of transcriptome in larvae development together with application of NGS techniques in the today aquaculture ended the general Introduction. In Chapter 2, the doctoral candidate puts forward audacious hypotheses concerning the influence of gamete quality, genetic (domestication, selection, maternal vs. paternal DNA), and environmental factors on the development and performance traits in Eurasian perch larvae and deliberately proposes pathways for their verification. These pathways are precisely described in the following four chapters (Chapters 3–6), which present independent experiments aimed at answering the candidate's research questions and verifying the hypotheses. This part of the dissertation already demonstrates the doctoral candidate's extensive theoretical knowledge of aquaculture, the European perch, and its role in the natural environment, as well as the challenges faced by farmers planning to rear this species under controlled conditions. Furthermore, Rosella Debernardis has demonstrated a thorough understanding of the current state of knowledge regarding molecular research on gametes and early developmental stages of fish, which enabled her to formulate the research objectives and the methodology for their implementation.

The chapters presenting the experimental designs and their results are prepared in the form of scientific manuscripts and consist of sections typical for such publications. The content of Chapter 3, entitled **Does transcriptome of freshly hatched larve describe past or predict future developmental trajectory**, has been published Aquaculture journal. The remaining three studies (chapter 4 – **Dynamic interplay of maternal and paternal contribution to offspring phenotype in Eurasian perch, *Perca fluviatilis***, chapter 5- **Transcriptome profile of fish eggs reflects embryonic survival potential, not larval fate**, and chapter 6 – **Heart oedema in freshly hatched larvae of Eurasian perch is associated with multi-tissue gene dysregulation**) are in preparation (chapter 5) or under review. This pivotal part of the dissertation is scientifically coherent and aligns well with its title. The designs of the subsequent experiments are sound and presented in a clear manner. Any doubts I may have had at this stage pertained to curiosity regarding the choice of a specific research path rather than any flaws in this regard. Rosella Debernardis conducted a series of scientific experiments, ranging from the incubation of fertilized eggs and larval rearing to assessment of perch gametes and larvae using microscopic and molecular techniques, supported by statistical tools. In all experiments, the doctoral candidate obtained results that allowed for the verification of at least some of the formulated hypotheses, which confirms ability of the doctorate candidate to conduct independent research. The format in which the results are presented is remarkably clear and easy to follow, despite the impressive volume of data. Obtaining such data required a significant investment of effort and time, as well as scientific inquisitiveness—the kind of commitment expected from the independent researchers! The results presented by Rosella provoke discussion, raise further questions, and are intellectually engaging! Below, I present how and to what extent the candidate has addressed the scientific hypotheses and objectives set out at the beginning of the dissertation.

Hypothesis 1: transcriptomic profiles of larvae can offer prediction concerning further fish development.

The title of the first chapter itself poses a question: **Does transcriptome of freshly hatched fish larvae describe past or predict future developmental trajectory?** Taking into account how easy in some cases is to affect gene expression and how transcriptome profiles change in time I would answer that transcriptome of freshly hatched larvae describe state of the larvae at the particular moment when they were sampled for RNA seq. But on a more serious note, I have to admit that in the recent years transcriptome of fish larvae has been examined in the very limited number of species excluding Eurasian perch. Only scientists from few laboratories have made the effort and perform similar analyses. This chapter is very extensive and contains research results regarding the relationship between performance/zootechnical traits of larvae at the various stages of larval development originated from each of the 16 families in European perch and the transcriptomic profile assessed at the mouth opening stage exclusively. Gene Ontology analysis was performed, hub genes were identified and key traits for aquaculture provided. With help of statistics and bioinformatics tools the PhD student found a relationship between the value of a given trait and a specific transcription pattern(s) in larvae. This discovery enabled conclusion that transcriptomic profile of perch larvae is a bridge between the past (parental gene expression during late embryogenesis) and the future – further after hatching development and may be considered as a the instruction for the larval period and potentially beyond. Moreover, according to the PhD student, such a link between breeding traits and the level of transcription of specific genes gives a chance to find molecular markers associated with the perch larval performance what may be used for the perch aquaculture. Nevertheless I have a doubt; I think you may be too optimistic about application of gene expression assessment in the context of the fish performance as the quantitative traits are encoded by dozens/hundreds of genes.

Hypothesis 2: larvae derived from two genetically distant parental individuals parental enable differentiation of maternal and paternal contributions.

Chapter 4: **Dynamic interplay of maternal and paternal contribution to offspring phenotype in Eurasian perch, *Perca fluviatilis***

In this research, a reciprocal crosses between wild and domesticated perch specimens were performed to analyse transcriptomic and phenotypic maternal and paternal contributions (at the level of gene transcription) to the offspring assessed at the mouth opening stage. This is a genuine approach that confirmed Rosella Debernardis has got good scientific theoretical and practical background to perform such research. To my knowledge similar experiments have been performed only in the limited fish species to date including zebrafish, medaka or *Megalobrama x Xenocypr* hybrids. Analysis of the chosen zootechnical traits in the larvae produced by the reciprocal wild x domesticated perch crosses exhibited dominant maternal effect on the offspring. Molecular examination actually has confirmed this observation. Transcriptome analysis supported by the bioinformatics and statistic tools showed significant transcriptomic diversity between different crosses with evident predominance of the maternal component! In my opinion, this is a really hot topic in the field of developmental biology! Maternal and paternal genomes cooperate but have different and specialized roles. For many years biologists dispute about equal genetic but different physiological contribution of two parents in mammals. Similar phenomenon has been observed in other vertebrates and plants. Studies on the *Xenopus* hybrids showed maternal dominance and paternal genes silencing. Results provided by Rosella may put new light on the evolution of reproductive modes in fish. Populations of some fish species are composed of only females that reproduce by natural gynogenesis (without contribution of paternal genome). Moreover, in fish it is

relatively easy to induce gynogenetic development while induced androgenesis (development of fish with only paternal nuclear genome) is not that efficient. Predominance of expression of maternal-originated alleles during early pre- and post-hatching development in fish may at least partially explain the unequal efficiency of gynogenesis and androgenesis.

Hypothesis 3: variability in egg quality, assessed through zootechnical traits (i.e., fertilization rate, embryonic developmental rate, etc.), is associated with distinct gene expression patterns that mediate maternal effects on larval development.

Chapter 5: **Transcriptome profile of fish eggs reflects embryonic survival potential, not larval fate**

Egg is a complete package containing everything what is crucial for the embryo development: maternally originated RNAs that control first 10-12 cell divisions, structure, and metabolism, acting as key instruction and fuel, with nutrients (lipids, proteins), vitamins, hormones, etc. supporting growth, and influencing development and embryonic behaviour. After MBT (mid blastula transition), new instruction originating from the parental genome appears. So, there is life before ZGA (zygotic genome activation) and after. Rosella asked questions about connection between egg quality and larval performance and application of that relationship (if observed). As in the previous studies, an effort has been made to employ RNA-seq to find here any transcriptomic differences between eggs and larvae with varied quality. And the results are very promising as she has found some variation of the gene transcription level between-groups and within-groups, especially in low and medium quality eggs. Moreover, larvae that developed in eggs with varied quality were found to exhibit similar transcriptomic profiles and comparable performance. Author of the thesis proposed in this chapter a concept of late egg developmental competence that may be considered as egg ability to develop normally from the fertilization up to after hatching early stages like tail detachment stage. One of the plots from this part of the thesis – connection between variation in the quality of eggs and variation in the egg transcriptomic profiles fits into the already established data on research trends aimed at searching for the markers of the fish egg quality and their application. Low quality eggs are considered those that are characterized by decreased survival rate, increased ratio of deformed larvae and sometimes increased of ploidy abnormalities. Many scientific teams have tried to develop reliable markers enabling discrimination high quality eggs from the low quality eggs in many aquacultured species. Some of them have succeeded...partially. It turned out that the obtained markers are almost always species specific, usually strain or stock specific and finally most of the approaches may be applied but only at the laboratory scale. Irrespective of the egg quality definition, it is tempting to study it but it is tough to get something reliable. Nevertheless, it is always a nice scientific journey. Though, the discussion and conclusions concerning connection between egg quality and larvae performance are in my opinion too preliminary in some parts, while in others are rather obvious; see comments below:

- Taking into account that instruction for the early embryogenesis appears during oogenesis in the oocyte cytoplasm and 50% of the larvae genome originates from the oocyte nucleus it is rather obvious that egg cargo affect not only embryonic stages but also every further step during the fish ontogeny.
- To have broader view of the issues related to the egg quality, examination of the egg maternal RNA is not enough. Assessment of at least some of the biochemical indices, activity of anti-oxidant enzymes, lipids, proteins, etc. could help to answer the doubts about inter-clutch variation in the egg developmental competence.

- The early versus the late egg development competence - maternal RNA versus parental RNA; different stages of development are governed by different instructions. So I am not that surprised that as you say “despite significant transcriptomic variation in unfertilized eggs, newly hatched larvae exhibited uniformity in both gene expression and zootechnical traits”.
- Have not you considered use of eggs from females that were not domesticated as controls for the experiment?
- Most of such experiments are terminated at the swim-up stage. From my experience, eggs with the higher survival at the eyed stage not always showed high survival after hatching, in the other cases after increased mortality before hatching, embryos that hatch develop further with any large declines. Have you continued your experimentation(s) to get juveniles or grown-ups?

Hypothesis 4: it is expected that transcriptomic comparisons between morphologically normal larvae and those exhibiting developmental anomalies, such as heart oedema will reveal key molecular pathways involved in abnormal development.

Chapter 6: Heart oedema in freshly hatched larvae of Eurasian perch is associated with multi-tissue gene dysregulation

This part of the thesis concerns perch larvae deformations. The background of the fish body deformations and malformations is complex and includes genetic factors (increase of the deformed larvae in the inbred strains), feeding, water quality or infections, among others. Here, the author has focused on the heart oedema, a deformation that is sometimes observed in large ratio in the aquacultured species what may cause economic losses. To date not some studies have been performed to understand factors that are responsible for formation of this malformation (actually most of the information about heart disease in fish originate from studies in zebrafish). That’s why results concerning genetic background of heart oedema disease in the perch larvae provided by Rosella need to be recognised as very important for developmental biology and the genetics of diseases of the farmed animals. To find out why heart oedema is formed in the perch, Rosella has engaged comparative transcriptomic analysis and bioinformatics of the well-formed larvae and those with the heart oedema. This is a proper and very logical scientific approach that was used before to examine pathological tissues in zebrafish and medaka among others. Sixteen heart specific transcripts were found by author of the thesis and further analysed by RT-qPCR. Results of RNA-seq and qPCR were confirmed in the case of seven transcripts. Enough to propose a scenario for the heart malformation and to find candidate genes for the further studies. I recon this part of the thesis very solid and convincing from the experimental design stage to the result analysis part. A very nice beginning for the further studies on the particular genes and their precise function. Only one comment – like above, sometimes author of the thesis has got too light hand for easily drawing conclusions, building multi story verbal structures in discussion and multiplying research hypotheses based on the restricted amount of precise data. But it does not decline at all the high quality of the entire chapter/paper.

Summarizing this part of the thesis, I need to emphasize Rosella’s great knowledge concerning connections between gene transcription of perch larvae, their performance and the traits that are important in terms of the fish breeding/rearing.

The dissertation concludes with chapters entitled General Discussion, Conclusions, Implications and Recommendations, as well as lists of tables, figures, and supplementary files, a

bibliography, and Authors' Statements. The attached Authors' Statements clearly indicate that the doctoral candidate's contribution to formulating the scientific assumptions, preparing and conducting the planned research, analysing the obtained results, and preparing the manuscripts was significant.

Evidently, the evaluation of the PhD thesis of Rosella Debernardis can only be positive. However, as a reviewer's duty apart from the specific comments I already made, I would like also to ask author of the thesis some questions concerning general issues that may be answered during the presentation of the assumptions and results of the doctoral thesis:

1. Role of the Eurasian perch in the European and Polish aquaculture - what is a true current status of perch in aquaculture? How much fish is produced in the aquaculture in comparison to perch caught by the fishery sector. Is there a real market for perch from aquaculture as cost of perch from wild stocks is still relatively low? Do you think the Eurasian perch from RAS may replace fish from the wild stocks?
2. Access to the domesticated perch gamete donors – transportation of eggs from distant places is a challenge sometimes. How egg transportation may affect gamete quality? Any perch stocks are available closer than Switzerland?
3. Gene expression is a complex process in which transcription and translation are independently controlled. In the dissertation, you have used “gene expression” as a synonym for “gene transcription”. It was because all the genes that transcription was examined are the ones that expression is mostly or even fully controlled at the transcription level?
4. RT-PCR and RNA-seq results – could you explain why these two techniques are sometimes not compatible in evaluation of the level of the gene transcription?

Final conclusion

After a critical review of the content of Rosella Debernardis's doctoral dissertation, I unequivocally state that the reviewed thesis meets all the requirements for doctoral dissertations as set forth in Article 187 of the Act of July 20, 2018 – The Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended). Therefore, I move to the Scientific Council of the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn to admit Ms. Rosella Debernardis to the subsequent stages of the doctoral proceedings.

Konrad Ocalewicz