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**Review of the doctoral dissertation of Rossella Debernadis entitled “*The transcriptome of newly hatched larvae as a window into the exploration of parental legacy and early life performance in Eurasian perch*”.**

### 1. Brief Summary of the Thesis

The PhD dissertation of Rossella Debernadis is based on an integrated experimental and analytical framework combining controlled experiments, laboratory-based analytical assays, and comprehensive transcriptomic analyses using a range of bioinformatic tools. The thesis consists of an introductory chapter, four manuscripts, two of which have been published (one recently), and a general discussion that goes beyond a narrative summary by integrating datasets from all four studies into a new overarching analysis, followed by concise conclusions and perspectives. Collectively, the chapters investigate the transcriptomic profile of Eurasian perch, with a particular focus on the mouth-opening stage coinciding with hatching, in order to evaluate its potential as a source of information on inherited traits and developmental potential.

The introductory chapter presents the Eurasian perch as a species targeted for the development of European aquaculture production and outlines key challenges related to reproductive efficiency and early developmental processes that influence production success. Particular attention is given to embryonic and larval stages, which represent critical bottlenecks in fish ontogeny. The chapter provides a morphological overview of larval developmental stages, with emphasis on key transitions around hatching, including mouth opening, first feeding, and swim bladder inflation. It then introduces the molecular pathways and conceptual approaches underpinning the subsequent studies, linking variability in early performance to heritable traits encompassing both genetic and non-genetic inheritance. In this way, the chapter establishes the biological, molecular, and conceptual framework for the transcriptomic and bioinformatic analyses presented in the thesis.

Building on this framework, the overall objective of the thesis is defined as determining what the larval transcriptome at the mouth-opening stage can reveal about parental legacy and subsequent performance in Eurasian perch. On the one hand, the studies aim to partition larval gene expression variance into maternal and paternal contributions, encompassing both genetic and non-genetic inheritance. On the other hand, they assess the predictive value of

transcriptomic signatures for early-life performance and aquaculture-relevant traits. The conceptual coherence of the thesis is established by outlining the four manuscripts and the hypotheses associated with each study.

Manuscript 1, *“Does transcriptome of freshly hatched fish larvae describe past and predict future developmental trajectories,”* examines whether the transcriptomic profile of Eurasian perch larvae at the mouth-opening stage reflects parental contributions and predicts subsequent larval performance. Sixteen larval families originating from distinct parental pairs were reared under standardised conditions, and detailed zootechnical traits were recorded throughout larval development up to 27 days post-hatch. RNA sequencing combined with weighted gene co-expression network analysis (WGCNA) revealed substantial transcriptomic variability among families and identified gene modules correlated with embryonic development, growth, survival, and aquaculture-relevant traits, highlighting the role of parental effects in shaping larval transcriptomes.

Manuscript 2, *“Dynamic interplay of maternal and paternal contributions to offspring phenotype in Eurasian perch (Perca fluviatilis),”* investigates the relative contributions of maternal and paternal effects using a reciprocal crossing design between wild and domesticated broodstock. By combining controlled pairwise crosses with transcriptomic profiling of larvae at the mouth-opening stage and detailed zootechnical assessments, the study disentangles parental influences on early performance. The results demonstrate a strong predominance of maternal effects across multiple traits, including hatching success, growth, feeding dynamics, and swim bladder inflation, while paternal effects were fewer and context dependent. Transcriptomic analyses identified both purely maternal-effect genes and a smaller set of conditionally paternal-effect genes.

Manuscript 3, *“Transcriptomic profile of fish eggs reflects embryonic survival potential, not larval fate,”* explores the relationship between egg quality, embryonic developmental competence, and subsequent larval performance. Using a commercial-scale fertilisation protocol with pooled sperm to minimise paternal effects, egg batches were classified based on embryonic developmental success beyond fertilisation. Transcriptomic profiling of unfertilised eggs and larvae at the mouth-opening stage, combined with larval rearing trials under separate and mixed culture conditions, showed that while egg quality strongly influences embryonic survival and hatching success, it does not reliably predict larval performance among viable larvae. These results support the concept that late embryonic developmental competence is a more meaningful predictor of larval quality than fertilisation rate alone.

Manuscript 4, *“Heart oedema in freshly hatched larvae of Eurasian perch is associated with multi-tissue gene dysregulation,”* investigates the molecular basis of heart oedema, a severe and lethal deformity observed in newly hatched larvae. Using a family-based design, transcriptomic profiles of larvae exhibiting heart oedema were compared with those of morphologically normal siblings at hatching. RNA sequencing revealed a strong separation between the two phenotypes, with thousands of differentially expressed genes. Subsequent tissue-specific analyses identified a limited set of heart-specific candidate genes primarily involved in glucose metabolism, oxygen transport, and cardiac structure, several of which were validated by qPCR. The study demonstrates that heart oedema is associated with a distinct transcriptomic signature and confirms its lethal nature at early larval stages.

The general discussion constitutes a major strength of the dissertation by providing an original, integrative synthesis of all transcriptomic and phenotypic datasets generated across the four studies. Structured around a “past, present, and future” framework, it links parental molecular legacy, the transcriptomic state at hatching, and subsequent developmental trajectories. By combining datasets, the discussion identifies a conserved core transcriptome at the mouth-opening stage alongside a smaller, context-dependent gene set shaped by parental effects and developmental perturbations. The chapter advances the concept of the larval transcriptome as both a molecular archive of parental effects and a predictor of later performance, thereby substantially enhancing the scientific contribution of the thesis. The dissertation concludes with brief final conclusions and perspectives.

## **2. Assessment of Theoretical Knowledge**

The dissertation clearly demonstrates the candidate’s solid theoretical knowledge across the disciplines relevant to the research performed, including physiology, molecular biology, and bioinformatics. The work is firmly grounded in experimental biology, using Eurasian perch as a model species with a clear focus on early life history and ontogeny, as well as parental effects.

The introduction, the four manuscripts, and the integrative synthesis presented in the general discussion collectively demonstrate in-depth understanding of the core theoretical concepts within these disciplines, as well as of complementary theoretical frameworks required to link molecular-level processes with organismal performance. All four manuscripts are built on sound theoretical foundations, particularly in physiology and molecular biology, and make effective use of advanced bioinformatic and analytical approaches that integrate biological and computational perspectives. The design of the studies and the interpretation of the results clearly rely on this theoretical competence, which is consistently demonstrated throughout the thesis. This is particularly evident in the candidate’s treatment of transcriptomic variance partitioning and in the interpretation of parental effects on larval quality at hatching and subsequent performance trajectories at both the molecular and organismal levels.

## **3. Assessment of Ability to Conduct Independent Research**

The dissertation clearly demonstrates the candidate’s ability to conduct independent scientific research. The work is characterised by an integrative approach that encompasses a wide range of scientific skills and responsibilities, collectively documenting a high level of research independence. As stated in the signed authorship declarations, the work performed by Rossella Debernadis includes conceptualisation, methodological development, investigation, data curation, formal analysis, visualisation, and the writing of original manuscripts.

These contributions comprise a comprehensive set of activities that together confirm the candidate’s ability to independently conduct complex scientific research. The investigative work includes substantial involvement in experimental design and in the execution of rearing operations related to fish reproduction and offspring culture, conducted with due consideration of animal welfare, as well as essential sampling procedures and zootechnical assessments. In the molecular laboratory, the extraction of RNA and the performance of qPCR analyses required additional technical expertise.

Data curation and formal analyses of transcriptomic datasets further involved differential gene expression and functional analyses, as well as the integration and biological interpretation of these data, which demands a distinct and advanced analytical skill set. Finally, the preparation

of the manuscripts demonstrates the candidate's ability to contextualise original findings within the relevant scientific literature and to communicate results clearly and coherently.

Considering the four-year doctoral research period, the scope and scientific depth of the four manuscripts, two of which have been published, as well as the comprehensive general introduction and the integrative general discussion, the dissertation provides clear evidence of the candidate's ability to conduct independent scientific research.

#### 4. Assessment of Originality

The dissertation constitutes an original approach to a well-defined scientific problem and leads to novel results with clear application potential and possible economic relevance. Focusing on Eurasian perch, a species of increasing interest for European aquaculture, the thesis addresses critical bottlenecks in early development by placing particular emphasis on the mouth-opening stage, a key transition coinciding with hatching and closely associated with the onset of exogenous feeding and swim bladder inflation. By targeting this specific developmental window, the work advances the concept of larval quality as a complementary and informative counterpart to traditional assessments of egg quality, thereby providing a novel perspective on early life performance and parental effects extending beyond embryonic genome activation.

A central element of the dissertation's originality lies in the application of transcriptomic approaches to this early larval stage in Eurasian perch, a species for which sufficiently annotated genomic resources enabling genome-wide transcriptomic analyses have only recently become available. By leveraging these emerging resources, the candidate applies state-of-the-art transcriptomic and bioinformatic analyses in a biological and applied context where such approaches have previously been limited, thereby enabling new insights into parental effects and the prediction of larval performance trajectories under defined conditions.

An additional aspect contributing to the originality of the dissertation is the timing of the research in relation to the availability of genomic resources for Eurasian perch. The reference genome for this species was initially annotated at the scaffold level in 2016 and subsequently improved to a chromosome-level assembly in 2020, at which point it became systematically informative for genome-wide analyses. As a result, comprehensive transcriptomic investigations in Eurasian perch have only recently become technically and analytically feasible and therefore remain relatively scarce in the literature.

Beyond its conceptual and methodological originality, the dissertation also opens avenues for original application of the results in a selective breeding context. In particular, the identification of consistent differences in early-life performance traits, such as swim bladder inflation efficiency among offspring derived from different parental backgrounds, illustrates how transcriptomic analyses can be used to explore the molecular basis of such variation. The genome-wide nature of the data provides a foundation for future genomic investigations aimed at identifying genes or regulatory pathways associated with improved larval performance, thereby offering clear potential for application in genomic or marker-assisted selection strategies in Eurasian perch aquaculture.

Taken together, the dissertation provides an original solution to a scientific problem through the novel integration of transcriptomic approaches, developmental biology, and aquaculture-relevant performance traits in a species-specific context, while also demonstrating original

application potential of the research results. On this basis, the originality criterion for the doctoral dissertation is clearly fulfilled.

## 5. Additional Remarks

The dissertation is generally very well structured and clearly written, with a coherent narrative that integrates experimental biology, molecular analyses, and transcriptomic data in a convincing manner. The language is precise and well formulated throughout the thesis, facilitating comprehension of both methodological and conceptual aspects of the work.

The referencing is appropriate and well selected, providing a solid basis in the acknowledged literature across the relevant disciplines. While the reference list is not intended to be exhaustive, it adequately covers the core literature required to support the theoretical framework, methodological approaches, and interpretation of results.

A few minor points could be considered for improvement. The introductory chapter could benefit from a brief overview of the development of genomic resources for Eurasian perch, including the progression from early scaffold-level assemblies to the availability of chromosome-level genome annotation. Such contextualisation would further clarify why genome-wide transcriptomic approaches have only recently become feasible for this species. In addition, the inclusion of a schematic illustration of larval ontogenetic stages, accompanied by precise information on developmental age and temperature regimes, could enhance clarity for readers less familiar with early Eurasian perch development.

Although ethical considerations are addressed, some procedural aspects could be described in greater detail. In particular, clarification regarding the use of anaesthesia during larval handling, the procedures for euthanasia in connection with sampling, and the fate of larvae following experimental measurements would improve transparency and completeness of the ethical documentation.

Finally, a small number of scientific points could be considered. Some limitations related to the experimental design in Manuscript 2 (the Dom–Wild study) could be discussed more explicitly, particularly with respect to potential differences in maternal hormonal treatment and egg transport. A clearer focus on the possible consequences of these factors, rather than downplaying their potential impact, would help to better delineate the scope and generalisability of the conclusions.

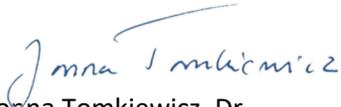
In addition, in a few instances alternative interpretations of specific results could be discussed in greater depth. For example, in Manuscript 1 (the larval transcriptome study), larvae of wild origin were on average the smallest at hatching but the largest at the end of the experiment. While this pattern may reflect compensatory growth, it could also be influenced by size-selective mortality, with higher mortality among smaller and weaker larvae. Addressing this possibility, for instance by considering effects on both average size and variability, would further strengthen the interpretation of the results.

Overall, these points are minor in nature and do not detract from the overall quality or conclusions of the dissertation but addressing them would further strengthen the discussion.

## 6. Final Recommendation

Taking into account the above considerations, I issue a positive assessment of the doctoral dissertation of Rossella Debernadis and recommend that the Scientific Council of the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn admit the dissertation to public defence and, following a successful defence, award Rossella Debernadis the doctoral degree in agricultural sciences in the discipline of animal science and fisheries.

Yours sincerely,



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Attachment 1:

Application for Distinction of the dissertation of Rossella Debernadis 2026-01-22 jt