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LECTURE 6

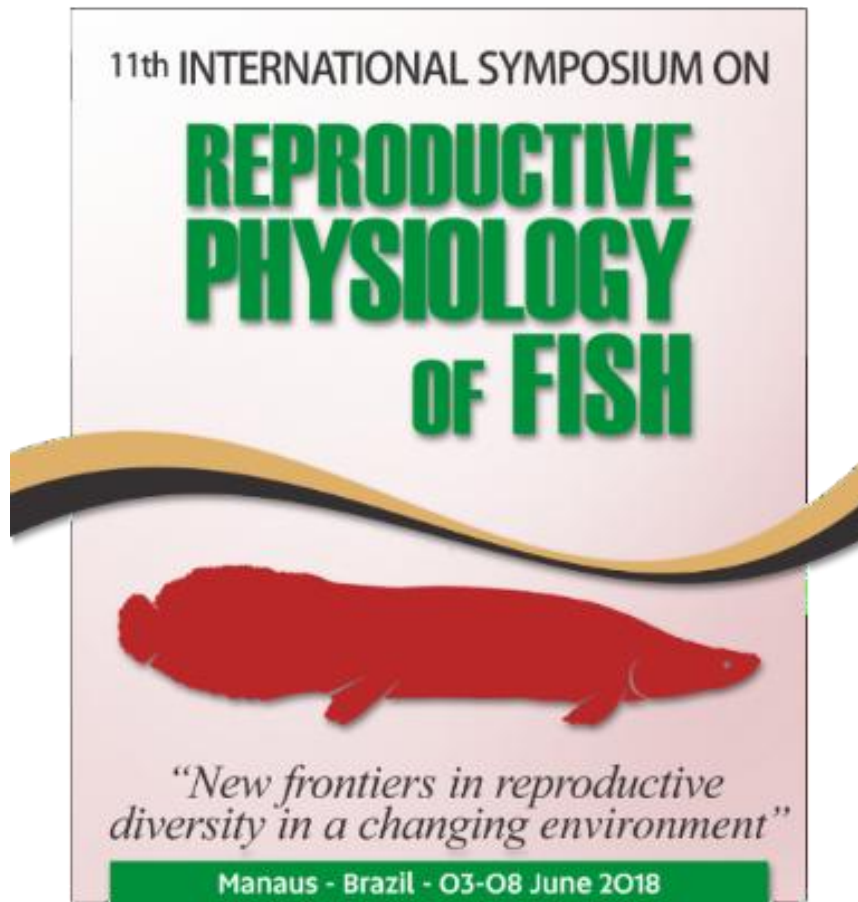
MOLECULAR PORTRAIT OF EGG QUALITY AND NOVEL MATERNAL-EFFECT GENES

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Good quality or developmentally competent fish eggs are successfully fertilized and develop normally as viable, non-malformed embryos. However, the detailed mechanisms involved in egg quality remain poorly understood and, at present, no predictive markers of egg quality exist. Early developmental success relies on maternally-inherited molecules deposited in the oocyte throughout oogenesis, and over the last few years, our work has aimed at drawing the molecular picture of poorly competent (i.e. bad quality) fish eggs in order to (1) further uncover the molecular mechanisms associated with low developmental success, (2) reveal new maternal-effect genes that play important roles during early development, and (3) possibly obtain molecular signatures based on maternal mRNA profiles in fertilized eggs that could be used as predictive markers of good quality eggs. A large transcriptomic analysis was performed in zebrafish (*Danio rerio*) using a high number of egg clutches obtained under normal husbandry conditions that reflect naturally occurring variability in egg quality. The differentially expressed gene profile revealed dysregulation of genes involved in translation and protein synthesis. Interestingly, translation was also one of the main processes dysregulated in a similar study conducted in Sea Bass (*Dicentrarchus labrax*). Analysis of a smaller number of zebrafish females that consistently produced bad eggs demonstrated a much lower variability in expression profiles that resulted in a higher number of differentially expressed genes identified between good and bad quality eggs. These indicate that while common/general traits of bad quality egg exist, a multiplicity of other factors can dysregulate specific processes that ultimately lead to reduced egg quality. To gain insight into their functions, genome editing using the CRISPR/Cas9 technology was performed to create mutants of two new potential maternal-effect genes, *otulina* (OTU deubiquitinase with linear linkage specificity a) and *slc29a1a* (solute carrier family 29, member 1a), that are especially abundant in the ovary. Due to the high efficiency of this technique, mutations could not be transmitted to the next generation and the phenotype was analyzed in F0 females. We observed that both *otulina* and *slc29a1a* mutant-derived eggs had very low developmental success. Eggs from mutant females crossed with *vasa:eGFP* males do not contain GFP, suggesting that they cannot be fertilized. These novel findings showed for the first time that *otulina* and *slc29a1a* are essential for the developmental competence of eggs, therefore, are crucial maternal-effect genes. Finally, we applied statistical modeling using Partial Least Square (PLS) regression and genetic algorithm to our data to search for possible molecular signatures. We observed the presence of strong gene signatures, which were statistically robust both in terms of reproducibility and validation by pseudo-data, to link gene expression to the survival rate of eggs in our transcriptomic data. In summary, the molecular mechanisms that control egg quality are complex and probably variable even though some core processes including translation and proteins synthesis appear to play a key role. Yet, some maternal-effect genes have been identified that are important for fertilization. Finally, it appears possible to statistically predict developmental success based on the molecular portrait of the egg.

PROGRAM AND ABSTRACTS



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