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## GENOME EDITING REVEALS DEVELOPMENTAL DEPENDENCIES ON SPECIFIC TYPES OF VITELLOGENIN IN ZEBRAFISH (*Danio rerio*)

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### Introduction

While multiplicity of vitellogenins (Vtgs) is the norm in fishes, it is usually unknown whether the different types of Vtg are required or have any unique functions. The objectives of this study were to discover which types of Vtg are essential, to discern when their yolk protein products are required during development, and to begin exploring their special physiological functions in the zebrafish, a model teleost expressing an especially diverse array of different forms of Vtg.

### Methods

A novel gene knock-out (KO) technology employing a multiple CRISPR approach was used to incapacitate all genes encoding *type-1 vtgs* (*vtg1*, 4, 5, 6, and 7) simultaneously, as well as *vtg3* individually. A chimeric gRNA system containing sgRNAs and nCas9n RNA was injected into one-cell stage embryos, which were subsequently screened for double strand breaks at defined target sites using conventional PCR. Detected mutations were confirmed by sequencing. Pure lines carrying desired mutations were produced by stepwise selection and pairing of mutated individuals at each generation. Loss of function of targeted genes was confirmed by real time quantitative PCR (qPCR), Western blotting (WB), and liquid chromatography tandem mass spectrometry (LC-MS/MS). Effects of treatments were detected by phenotypic observations.

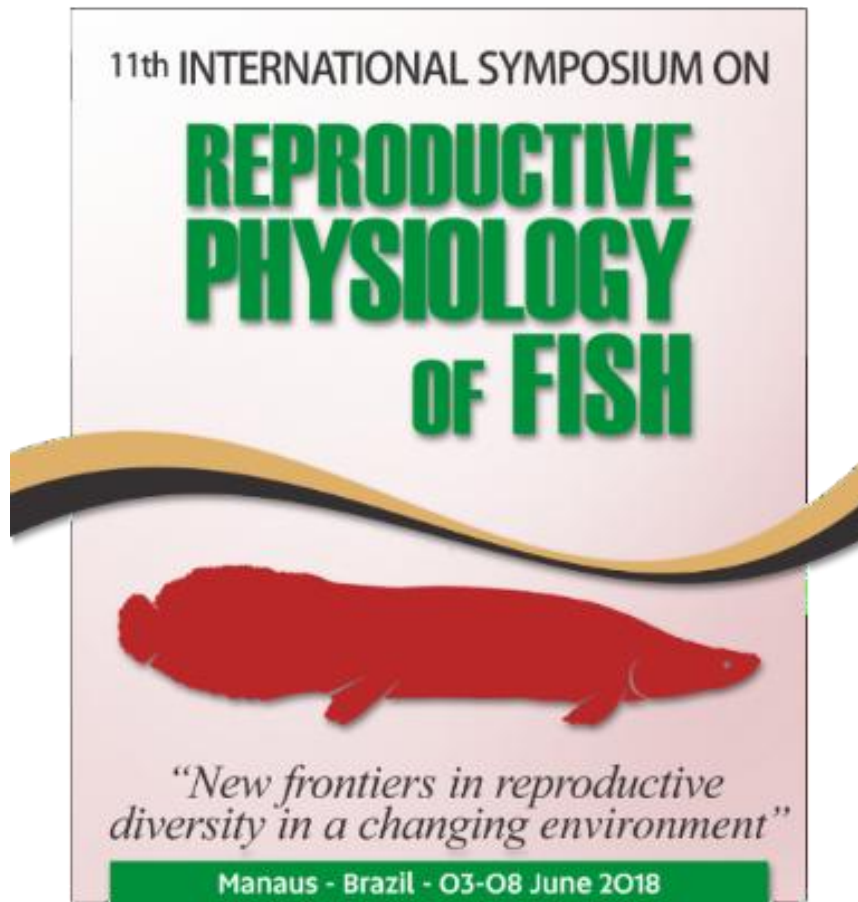
### Results and Discussion

Mutations of large deletions (1281-1182 bp) on gDNA for *type-1 vtgs*, and also for *vtg3*, resulted in frame shifts of their polypeptide sequences. qPCR, WB and LC-MS/MS results proved the collective KO of *vtg1*, 4 and 5, and the possible escape of *vtg6* and *vtg7* from Cas9 editing. The same type of analyses confirmed complete incapacitation of the *vtg3* gene. Both KO of *type 3 vtg* and *type 1 vtgs* severely altered survival and development of the respective 4<sup>th</sup> generation, pure line, mutant progeny. The KO of *vtgs1*, 4 and 5 resulted in a major decrease in embryonic survival at 24h, with 100% larval mortality by day 15; KO of *vtg3* resulted in a sharp decrease in embryonic survival by 8h, with a mean larval survival of only 6.25 % by day 22. Remarkably, in both cases, evidence for a compensatory increase in Vtg7 levels was observed. KO of *type 1 vtgs*, and also of *vtg3*, caused deformities such as cardiac and abdominal edema and spinal cord defects at 100 % and 30 % frequencies by day 11, respectively.

### Conclusion

The KO of multiple genes encoding different forms of Vtg was achieved, for the first time in any vertebrate, using the zebrafish model. Protein products of collectively knocked-out *vtg1*, *vtg4*, and *vtg5*, are required for late embryonic and larval development and survival. Vtg3, the least abundant form of Vtg in zebrafish and one thought to be mainly a nutrient for late stage larvae in other species, clearly has essential functions in early embryonic development, during body axis formation. Finally, the up-regulation of Vtg7 at transcript and protein levels in *vtg* KO fish may be part of a previously unknown system for regulation of vitellogenesis, a phenomenon which begs further investigation.

# PROGRAM AND ABSTRACTS



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