



EMBRYONIC STAGES OF SPOTTED GREEN PUFFERFISH (*TETRAODON NIGROVIRIDIS*) AS AN EXPERIMENTAL MODEL ORGANISM

Türker Bodur^{1,4*}, Andreas Zaucker^{2,4}, Jochen Gehrig^{3,4}, Yavor Hadzhiev⁴, Craig Watson⁵,
Ferenc Müller⁴

¹ Department of Aquaculture, Fisheries Faculty, Akdeniz University, Antalya, TURKEY. ² Cell and Development Biology, Warwick Medical School, University of Warwick, Coventry, UNITED KINGDOM

³ Acquirer AG, Sophienstraße 136, 76135 Karlsruhe, GERMANY.

⁴ Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, U.K.

⁵ Tropical Aquaculture Laboratory, Program in Fisheries and Aquatic Sciences, School of Forest Resources and Conservation, Institute of Food and Agricultural Sciences, University of Florida, Ruskin, Florida. U.S.A.

*Corresponding author turkerb@akdeniz.edu.tr

Abstract

Spotted green pufferfish *Tetraodon nigroviridis* (also known: *Dichotomyctere nigroviridis*, Marion de Procé, 1822) is prevalent in the rivers, estuaries, mangroves, and seas of Southeast Asia, where it can attain a length of 17cm. It is a popular aquarium fish, best reared in brackish water. In the 1960's, an extensive survey of nuclear DNA content in a range of teleost fish showed that species of the Tetraodontidae family, including spotted green pufferfish, possess the smallest genomes of all vertebrates known to date. Their compressed genomes are thought to be enriched for functional DNA compared to larger vertebrate genomes, and they are important models for comparative genomics. However, there is only a very limited utilization of pufferfish as an experimental model organism, due to the lack of established husbandry and developmental genetics protocols. In this study, we report to our knowledge the first *T. nigroviridis* stage series of embryonic development. Embryos were obtained by in vitro fertilization of eggs, and subsequent development was monitored by brightfield microscopy at constant temperature. Embryo yolk is transparent and contains an estimated 300–400 oil droplets varying in size (23.61–138.60 µm in diameter). Tetraodon embryonic development followed recognizable periods similar to the ones in zebrafish, including zygote (~2min), cleavage (~5h), blastula (~6h 30min), gastrula (~9h), segmentation (~14h 30min), pharyngula (~1d 19h) and hatching (~3h) periods. We also provided a brief description of the first 3 days of postembryonic development. The early developmental stage series described in this study give important basic information for developing reporter assays to test genomic elements or for molecular analysis of development by in situ approaches. Furthermore, we define developmental stages for future genome and transcriptome analysis also for its culture activities.

Keywords: *Tetraodon nigroviridis*, embryonic development, pufferfish, model organism