

USING DIFFERENT INCUBATION CONDITIONS TO CONTROL THE EMBRYONIC DEVELOPMENT OF THE ANNUAL FISH *Nothobranchius furzeri*, AN EMERGING VERTEBRATE MODEL FOR TOXICOLOGICAL RESEARCH

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INTRODUCTION: The annual fish *Nothobranchius furzeri* has emerged as a promising model for toxicological studies due to its unique life cycle characteristics, such as 1) drought-tolerant embryos that endure total desiccation of the temporary pools where it can be found, as they enter diapausing states, and 2) the fastest growth and aging rates ever described for a cultivated vertebrate. Additionally, one of its main advantages is the possibility to manipulate embryonic development through temperature control, enabling targeted studies of specific developmental stages. The formation of a golden iris is a key morphological marker that indicates the final stage of successful embryonic development. *N. furzeri* has been recently implemented in our laboratory as an ecotoxicological model, but studies on cultivation conditions are lacking.

OBJECTIVE: To evaluate how different incubation temperatures and hydrologic conditions (dry and aqueous) affect the viability and developmental rate of *N. furzeri* embryos.

MATERIALS AND METHODS: *N. furzeri* embryos were incubated under three temperature conditions: 18°C, 27°C, and room temperature (24°C average). Post-fertilization embryos were maintained in Petri dishes with two substrates: dry (moistened coconut fiber) and aqueous. For each condition, 10 embryos were used. Observations were conducted daily over a 60-day period using a stereomicroscope to monitor development and viability. Mortality was assessed based on absence of cardiac activity and loss of characteristic pigmentation.

RESULTS AND CONCLUSION: Embryos incubated at 27°C in an aqueous environment showed no mortality and consistent formation of the golden iris as early as 12 days post-fertilization, suggesting diapause skipping and making it the ideal maintenance condition for immediate toxicological studies. Cultivation in both water and dry substrate at 18°C elicited slow development due to obligatory diapause, as no animal passed the somitogenesis stage at 60 days post-fertilization. Thus, maintenance at 18°C is indicated for long-term embryo storage. These findings reinforce the versatility of *N. furzeri* as an effective tool in toxicology and developmental biology. Potential applications include 1) studying diapause in vertebrates; 2) assessing environmental stress; and 3) unveiling mechanisms related to aging and transgenerational toxicological effects.

KEYWORDS: Dormant embryos, Ecotoxicology, Thermal adaptability, Experimental models.

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