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Embryonic Developmental Response of Nile Tilapia (Oreochromisniloticus) to Different Aquatic Incubation Media

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Abstract
This study examined the comparative effects of pond water and rice field water on the embryonic development and larval viability of Nile tilapia (Oreochromis niloticus). A total of 300 fertilized eggs were incubated under controlled laboratory conditions using two distinct water sources, each replicated three times. Embryonic development was observed microscopically at 30-minute intervals, with documentation of cleavage, blastula, gastrula, organogenesis, and hatching stages. The results showed that embryos incubated in rice field water experienced significant developmental delays, irregular morphogenesis, and lower hatching rates (73.2%) compared to those in pond water (98.7%). Larvae from the rice field water group exhibited reduced motility, abnormal morphology, and higher post-hatching mortality. These adverse effects were attributed to physicochemical stress factors such as reduced dissolved oxygen, pH fluctuation, and potential agrochemical contaminants in rice field water. The study underscores the importance of water quality management in hatchery operations and recommends pretreatment or quality assessment of rice field water prior to use in aquaculture, especially in integrated farming systems.

INTRODUCTION

Aquaculture has become an essential component of global food security, particularly in low- and middle-income countries where the demand for affordable and sustainable sources of animal protein continues to rise (Maulana et al., 2023). Among freshwater aquaculture species, Oreochromis niloticus (*Nile tilapia*) holds a dominant position due to its fast growth rate, resilience to diverse environmental conditions, and wide market acceptance. In Indonesia, tilapia farming plays a significant role in supporting rural economies and contributing to national fishery outputs. However, the success and sustainability of tilapia aquaculture are closely linked to the quality of early developmental stages, especially embryogenesis, which determines larval viability, physiological robustness, and overall survival potential (Wardiman, 2019).

Embryonic development in teleost fish progresses through several well-defined stages: fertilization, cleavage, blastulation, gastrulation, and organogenesis (Arif et al., 2025; Raharjeng et al., 2022). These phases are characterized by rapid cell division, differentiation, and morphogenetic movements essential for the proper formation of major organs such as the brain, heart, and gills. The efficiency and success of these developmental processes are highly dependent on external environmental factors, including temperature, dissolved oxygen levels, pH, and the chemical composition of the incubation water.

Disruptions in these factors can lead to delayed development, morphological deformities, or embryonic mortality (Gutierrez-Lovera et al., 2019; Yunita et al., 2024). As such, understanding how environmental conditions influence embryogenesis is crucial for optimizing hatchery management and improving production outcomes (O'Rourke, 2019; Rola et al., 2021; Takai et al., 2020).

In many conventional hatcheries, pond water is the preferred medium for egg incubation due to its relatively stable physicochemical properties (Yudha et al., 2025). However, in rural and agricultural regions of Indonesia, small-scale fish farmers often utilize rice field water for incubation because of its ease of access and minimal cost. Compared to pond water, rice field water typically exhibits higher turbidity, elevated organic content, greater microbial load, and the presence of agricultural runoff, which may include agrochemicals (Anggraini, 2020; Arif et al., 2025; Soegianto, 2023). These differences may adversely affect embryonic development, yet few empirical studies have directly compared the effects of these two water sources on tilapia embryogenesis under controlled laboratory conditions.

Previous research has shown that poor-quality or unstable aquatic environments can induce a range of developmental anomalies in fish embryos, such as delayed cleavage, abnormal somite formation, spinal malformations, pericardial edema, and incomplete organogenesis (Adaninggar et al., 2024; Zizioli et al., 2023). These conditions are often linked to hypoxia, pH instability, and exposure to chemical stressors like nitrates and pesticides. Such abnormalities not only compromise hatching success but also reduce larval fitness and survival rates, posing a serious threat to the sustainability of aquaculture operations.

Despite the growing use of rice field water in aquaculture, particularly in integrated rice–fish farming systems, the impact of this water source on the embryonic development of O. niloticus remains insufficiently explored. Addressing this knowledge gap is essential for developing evidence-based strategies to enhance hatchery practices in diverse environmental settings.

Therefore, this study aims to examine the influence of different incubation water sources pond water and rice field water on the embryonic development and larval viability of Nile tilapia. Specifically, it seeks to: (1) compare the developmental progression of O. niloticus embryos in both water types; (2) assess differences in hatching success and morphological integrity; and (3) identify developmental anomalies associated with each environment. The results are expected to contribute valuable insights for improving water quality management in hatchery operations and promoting environmentally adaptive aquaculture systems in rural settings.

METHOD

Location and Duration

This study was conducted at the Fish Seed Center (Balai Benih Ikan) in Tanah Datar Regency, West Sumatra, Indonesia, from April 25 to May 14, 2025. All embryonic development observations were performed at the Zoology Laboratory, Department of Biology, UIN Mahmud Yunus Batusangkar, under controlled laboratory conditions suitable for accurate microscopic monitoring of embryogenesis in Oreochromis niloticus.

Materials and Equipment

The primary materials included fertilized eggs of Oreochromis niloticus obtained from naturally spawning broodstock. Two types of water were used as incubation media: pond water and rice field water, each representing common aquatic environments in rural aquaculture. Each treatment used three replicates, with 50 fertilized eggs per replicate, totaling 150 eggs per treatment. A detailed list of materials and equipment is provided in Table 1.

Table 1. Materials and Equipment

Materials	Equipment
Fertilized O. niloticus eggs Pond and rice field water	pH Meter Incubation Tank Olympus Inverted microscope Stopwatch

Data Collection

Embryonic development was monitored using an Olympus inverted microscope at 30-minute intervals. Observations began immediately after fertilization and continued through cleavage, blastula, gastrula, organogenesis, and hatching. Any morphological anomalies or delays were recorded and documented photographically. The time required to reach each developmental stage was noted for each treatment group. Water quality was recorded twice daily (morning and afternoon), and averages were calculated to evaluate differences between treatments.

Experimental Design

This study employed a descriptive observational design with replication. Each treatment (pond water and rice field water) was replicated three times to ensure consistency and reliability of results. Fertilized eggs were randomly assigned to each replicate.

Data Analysis Procedure

Healthy broodstock fish were allowed to spawn naturally under controlled conditions during the night. Fertilized eggs were collected the following morning with care to prevent damage. The eggs were then incubated separately in pond water and rice field water containers. The data collected included: Timing of developmental stages (hours post-fertilization), Percentage of successful hatching per replicate, Observed abnormalities (qualitative), Larval viability post-hatching (percentage survival 24 hours after hatching), Water quality parameters (DO, pH, temperature, turbidity, nitrate). Descriptive statistics were used to summarize developmental timing and hatching performance.

RESULT AND DISCUSSION

This study investigated the embryonic development and early larval viability of Oreochromis niloticus reared in two aquatic environments: controlled pond water and rice field water. Although both groups of embryos completed all fundamental developmental stages, distinct differences were observed in the timing and outcomes of development. Embryos in pond water progressed through cleavage, morula, blastula, gastrula, organogenesis, and neurulation in a timely and orderly manner (see figure 1). In contrast, embryos in rice field water showed consistent delays of approximately five to ten minutes per stage, indicating that the water quality in rice fields may not be optimal for supporting rapid and stable embryogenesis.

Microscopic observations conducted at 30-minute intervals revealed detailed progression through each developmental stage. During the cleavage stage (0.5 hours post-fertilization), symmetrical blastomeres formed in both groups, but division in rice field water appeared slightly delayed and less regular. At the morula stage (1.0 hpf), blastomeres in pond water were compact and clearly defined, while those in rice field water appeared loosely organized. The blastula stage (1.5 hpf) in pond water showed an evenly expanding blastoderm, whereas in rice field water, the blastoderm expansion was slower and uneven. Gastrulation (2.0–2.5 hpf) commenced with the formation of the embryonic shield in both environments, but the shield was more clearly defined in pond water embryos. Organogenesis (3.0–3.5 hpf) marked by somite formation and neural fold development was more advanced in pond embryos, while those from rice field water showed reduced axial clarity and delayed segmentation.

The observed developmental delays in rice field water can be attributed primarily to physicochemical stress. Embryogenesis is a highly energy-dependent process that relies on efficient mitochondrial ATP production to support rapid mitosis and cellular differentiation (Jia et al., 2019; Laisk et al., 2019; Zhang et al., 2022). Environmental parameters such as dissolved oxygen concentration, pH, ionic balance, and turbidity directly affect these processes. In rice field water, microbial activity and the decomposition of organic material may reduce oxygen availability and increase turbidity, compromising oxidative phosphorylation (Brinkmann, Schneider, Bluhm, 2019; Malafaia et al., 2020). Additionally, unstable pH and osmotic stress may activate compensatory cellular responses that slow development (Dadiono & Widodo, 2022; Jia et al., 2019; Nurlian, 2020; Tschirren et al., 2018).

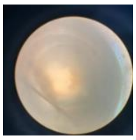
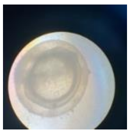
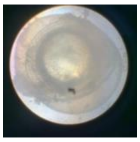
Time Post Fertilization	**Pond Water Image**	**Stage (Pond)**	**Rice Field Water Image**	**Stage (Rice Field)**
1.5 hours		Blastula		Early Blastula
2.0 hours		Gastrulation		Blastula
4.0 hours		Organogenesis		Gastrulation
5.0 hours		Tail Bud Stage		Organogenesis
6.0 hours		Pre-Hatching		Pre-Hatching

Figure 1. Embryogenesis of Nile Tilapia (*Oreochromis niloticus*) in Pond and Rice Field Water

Beyond physical parameters, the presence of agrochemical contaminants in rice field water represents another significant factor that may impair development. Residues of herbicides, pesticides, and fertilizers such as glyphosate, organophosphates, and triazoles have been reported in agricultural runoff and have known teratogenic and cytotoxic effects on aquatic embryos (Cormier et al., 2022; Malafaia et al., 2020; Muliari et al., 2020; Pereira et al., 2020; Takai et al., 2020; Yunita et al., 2024). These chemicals can disrupt transcriptional regulation, endocrine signaling, and induce oxidative stress, thereby interfering with critical processes such as somitogenesis and neurulation (Piña et al., 2018).

Table.2 Hatching rate, abnormality rate, and larval survival of Nile Tilapia (*Oreochromis niloticus*) in Pond and Rice Field Water

Parameter	Pond Water (%)	Rice Field Water (%)
Hatching rate	98.7 ± 1.2	73.2 ± 3.4
Abnormal larvae	2.0 ± 0.5	24.7 ± 2.1
Larval survival (24 h post-hatch)	96.5 ± 1.4	68.3 ± 2.8

Based on Table 2. embryos in rice field water eventually hatched, larval viability was significantly reduced. Larvae from pond water were active, morphologically normal, and exhibited near 100% survival. In contrast, many larvae from rice field water displayed abnormal curvature, weak motility, or died shortly after hatching. These symptoms indicate cumulative physiological stress throughout development. Furthermore, the absence of beneficial microbial communities or plankton in rice field water may deprive larvae of microbial protection and early nutritional support, compounding their vulnerability (Lotfy et al., 2022; Muliari et al., 2019; Namiki et al., 2018; Soleh et al., 2023). Such deficiencies highlight the limitations of using untreated rice field water in hatchery settings.

The implications of these findings are particularly important for integrated rice-fish farming systems, which are often promoted for sustainability in aquaculture. While the use of rice field water might appear cost-effective and environmentally integrated, this study demonstrates the need for critical water quality management when such systems are applied to early fish development stages. The presence of sublethal but chronic stressors in rice field water whether chemical, microbial, or physical can significantly impair both the speed and success of embryogenesis, ultimately affecting productivity (Nguyen et al., 2021; Sucipto, 2024). As a precautionary measure, water quality monitoring, filtration, or treatment should be implemented when rice field water is considered for hatchery use. Future research should aim to identify threshold levels of specific contaminants and design scalable intervention strategies to improve the compatibility of rice-based systems with fish breeding operations. Moreover, the developmental plasticity observed in embryos reared in rice field water may offer insight into environmental adaptability but must be interpreted with caution (Khosim et al., 2023; Moura et al., 2021; Rahman et al., 2020). While some embryos survived despite unfavorable conditions, the high mortality rate and developmental delay suggest that only a fraction of individuals can tolerate such stress, potentially leading to reduced genetic diversity and growth performance in subsequent life stages (Merola et al., 2020; Watt et al., 2021). This underscores the importance of evaluating not only short-term developmental success but also long-term viability and adaptability in suboptimal aquaculture environments (Adaninggar et al., 2024; Nurlian, 2020; Soegianto, 2023).

In future research, molecular approaches such as gene expression profiling or stress biomarker assays could further elucidate the specific developmental pathways affected by rice field water exposure. Identification of upregulated stress response genes, disrupted morphogenetic signaling, or altered metabolic activity could provide mechanistic insights into the precise cellular disruptions occurring during early embryogenesis. Such information would be valuable for developing targeted water treatment technologies or selecting more resilient broodstock strains suitable for integrated rice-fish systems.

CONCLUSION

This study confirms that water quality significantly influences the embryonic development and larval viability of Oreochromis niloticus, with pond water supporting faster development, better morphology, and higher survival compared to rice field water. The developmental delays and abnormalities observed in rice field water are likely caused by physicochemical stress and possible agrochemical exposure. Although informative, the study is limited by a small sample size and the absence of detailed water chemistry data. Future research should include water quality profiling and evaluate specific interventions such as mechanical filtration or safe chemical treatments to improve hatchery outcomes in integrated rice–fish farming systems.

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