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OPTIMIZING THE BREEDING AND REARING TECHNIQUES OF TRANSGENIC GLOFISH TETRA (*Gymnocorymbus ternetzi*) IN TROPICAL FRESHWATER AQUACULTURE SYSTEMS

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ABSTRACT

The transgenic GloFish Tetra (*Gymnocorymbus ternetzi*), engineered to express fluorescent proteins, is increasingly popular in the global ornamental aquaculture industry. However, optimized protocols for its breeding and larval rearing under tropical freshwater conditions remain underexplored. This study aimed to evaluate the effects of substrate type and larval stocking density on reproductive performance, larval survival, growth, feed utilization, and fluorescent color intensity in GloFish Tetra. A 3×3 factorial experiment was conducted at a tropical hatchery in Indonesia using three substrate types—no substrate (S0), artificial fiber mat (S1), and natural aquatic vegetation (Hydrilla verticillata, S2)—combined with three stocking densities: 15, 10, and 5 larvae per liter. Key parameters, including fecundity, fertilization rate, hatchability, survival rate (SR), specific growth rate (SGR), feed conversion ratio (FCR), and color intensity (ΔE), were measured and statistically analyzed using a two-way ANOVA. Results demonstrated that the S2-D5 treatment (natural vegetation with low density) significantly improved fecundity (330.2 ± 10.8 eggs), hatchability (82.3 ± 2.7%), SGR (4.30 ± 0.11%/day), and color intensity (ΔE = 17.6 ± 1.0), while achieving the lowest FCR (1.31 ± 0.07). In contrast, high-density and substrate-free conditions (S0-D15) yielded the poorest performance across all metrics. These findings underscore the crucial role of environmental enrichment and density management in enhancing both biological and aesthetic traits in GloFish Tetra. This study presents a validated protocol for enhancing hatchery success and improving the ornamental quality of transgenic fish in tropical aquaculture systems.

Keywords: GloFish Tetra, ornamental aquaculture, stocking density, spawning substrate, larval survival, fluorescent protein expression, tropical hatchery

INTRODUCTION

Aquaculture has evolved into one of the most dynamic and fastest-growing sectors in global food and non-food production. While historically focused on meeting protein demands through food fish, modern aquaculture has diversified into ornamental species, which now constitute a significant niche market. The global

ornamental fish trade is valued at over USD 6 billion, with annual growth rates exceeding 8%, driven by expanding consumer interest, advances in breeding technology, and globalized trade networks (Mandrioli et al., 2022). Among the most striking developments in this sector is the emergence of transgenic ornamental fish, notably GloFish®, which are genetically modified to express fluorescent proteins derived from marine organisms such as jellyfish and coral (Deich et al., 2023; Leggatt & Devlin, 2020). These fish offer unique aesthetic appeal and are increasingly popular in home aquaria and educational exhibits worldwide.

One of the leading transgenic varieties is the GloFish Tetra (*Gymnocorymbus ternetzi*), a genetically engineered form of the black skirt tetra. GloFish Tetra express vivid, heritable fluorescence under specific light spectra and are designed for ornamental purposes only, not for consumption (Cox & Fitzpatrick, 2023; Wang et al., 2023). Regulatory frameworks governing transgenic aquatic species vary globally. However, GloFish have been legally approved for cultivation, distribution, and sale in several countries, including Indonesia, where ornamental aquaculture is experiencing rapid development (Hoseinifar et al., 2023; Tarihoran et al., 2023). Indonesia's tropical climate, biodiversity, and potential for rural aquaculture make it an ideal hub for scaling transgenic ornamental fish farming (Jerikho et al., 2023; Syandri et al., 2023). However, the scientific understanding of optimal husbandry protocols for these genetically modified fish in tropical aquaculture systems remains underdeveloped.

While GloFish Tetra share many biological traits with their wild-type progenitors—being small, egg-scattering characins with relatively fast reproductive cycles—they may exhibit distinct physiological responses due to their transgenic nature (Meirelles et al., 2021; Sun et al., 2020). For instance, the metabolic costs of expressing fluorescent proteins, sensitivity to environmental stressors, and pigmentation pathways may differ from their non-modified counterparts (Alfakih et al., 2022; Borbély et al., 2022; He et al., 2021). These differences necessitate the customization of breeding and rearing strategies explicitly tailored to the biology of transgenic fish, rather than assuming equivalency with wild-type species (McClelland et al., 2020; Wu et al., 2022). Unfortunately, many small-scale hatcheries in Southeast Asia rely on empirical or trial-and-error approaches, often lacking standardized guidelines, which can lead to suboptimal reproductive success, reduced larval viability, and inconsistent color expression, thereby reducing market value.

The reproductive performance of GloFish Tetra is highly dependent on environmental cues such as substrate type, photoperiod, and water temperature, factors known to influence oviposition in characin fishes. However, empirical data on the effectiveness of various spawning substrates (e.g., artificial mats vs. natural aquatic vegetation) under tropical hatchery conditions remain scarce. Additionally, larval rearing faces challenges associated with stocking density, feed management, and water quality stability, all of which impact key performance metrics such as survival rate, specific growth rate (SGR), feed conversion ratio (FCR), and fluorescence intensity—a critical aesthetic trait in the ornamental fish industry (Ballesteros-Redondo et al., 2023; Sen Sarma et al., 2023; Swain et al., 2022).

Another concern specific to transgenic species is the stability of phenotypic traits, including the consistency of fluorescence over time. Research suggests that environmental stress, such as temperature fluctuations, oxidative stress, or nutritional deficits, may suppress the expression of fluorescent proteins or lead to developmental abnormalities (Plagens et al., 2021; Rodrigues et al., 2022; Sewelam et al., 2020). Therefore, optimizing rearing conditions that minimize physiological stress is essential

not only for survival and growth but also for maintaining marketable coloration. Moreover, in tropical aquaculture systems—characterized by elevated ambient temperatures, fluctuating dissolved oxygen levels, and variable filtration practices—these stressors are even more pronounced and warrant dedicated investigation (Dettleff et al., 2022; Song et al., 2022).

To date, few studies have systematically assessed the breeding and rearing of GloFish Tetra under tropical freshwater conditions. The majority of knowledge originates from controlled laboratory environments in temperate regions, which do not account for the realities of low- to middle-income aquaculture systems. There is thus a critical need to develop evidence-based, field-applicable protocols that reflect the environmental, technical, and economic constraints of tropical ornamental hatcheries. Variables such as substrate type, stocking density, feed regimen, and light regime require empirical assessment to optimize the performance of transgenic tetras while ensuring animal welfare, aesthetic quality, and cost efficiency.

Indonesia, as one of the world's leading aquaculture-producing nations, provides a pertinent setting for such research. At sites such as Andar Farm in Lingsar, West Lombok, field-based experimentation can generate data that is both scientifically robust and practically relevant. Through rigorous experimentation, it is possible to determine best-practice protocols that not only enhance biological performance (e.g., growth and survival) but also improve the visual and commercial quality of ornamental transgenic fish. Therefore, the present study aims to: (1) optimize the spawning and larval rearing protocols of transgenic GloFish Tetra (Gymnocorymbus ternetzi) in tropical freshwater aquaculture systems, and (2) Evaluate the effects of environmental enrichment (via spawning substrate) and stocking density on reproductive performance, larval growth, feed utilization, and fluorescence expression.

The findings of this study are expected to make a significant contribution to the development of scalable, cost-effective, and environmentally sustainable aquaculture techniques for transgenic ornamental fish. Furthermore, they offer valuable insights into how genetic and environmental interactions affect aquaculture outcomes in resource-limited tropical contexts, thereby informing policy, extension services, and smallholder innovation in ornamental fish farming.

MATERIALS AND METHOD

2.1 Experimental Site and Design

This study was conducted over 60 days (March–May 2025) at the Andar Farm Experimental Hatchery Unit in Lingsar, West Lombok, Indonesia. The experimental design followed a 3 × 3 full-factorial layouts, integrating three substrate types—no substrate (S0), artificial polyester fiber mats (S1), and natural aquatic vegetation (*Hydrilla verticillata*, S2)—with three larval stocking densities: 15 larvae/L (D15), 10 larvae/L (D10), and 5 larvae/L (D5). This yielded nine distinct treatment combinations, each replicated three times (n = 3), resulting in a total of 27 experimental units. Each replicate was housed in a 20-liter glass aquarium, maintained under controlled and uniform physicochemical conditions.

2.2 Broodstock Management and Spawning Procedures

A total of 81 sexually mature GloFish Tetra (*Gymnocorymbus ternetzi*), aged 6–8 months (average weight: 0.85 ± 0.09 g), were used as broodstock. The fish were randomly allocated into 27 spawning groups, each consisting of 1 male and two

females, based on the ideal sex ratio for black skirt tetra spawning (Volpato et al., 2021). Broodstock conditioning took place in 60-L fiberglass tanks for 14 days under a controlled photoperiod of 14 hours of light and 10 hours of darkness, implemented via automated LED light timers (Eletech 24-Hour Digital Timer). The ambient water temperature was maintained at 27 ± 1°C, with a pH of 7.0 ± 0.2, and dissolved oxygen (DO) levels above 6.0 mg/L, consistent with ornamental broodstock management standards (Boyd & Tucker, 2012).

Fish were fed twice daily (08:00 and 17:00) with a mixed diet: live *Tubifex* worms and a high-protein commercial ornamental feed (Tropical Discus Gran D-50 Plus, Poland) containing 45% crude protein, 8% fat, 2% fiber, and supplemented with astaxanthin and spirulina to enhance color expression and gamete quality. On the evening of the 15th day, broodstock groups were transferred into experimental spawning tanks with the assigned substrate treatments. The next morning, adult fish were removed to prevent egg predation, and fertilized eggs were collected using a gentle siphoning technique. Eggs were rinsed with aged water and incubated in 1-L static tanks dosed with 0.2 ppm methylene blue to suppress fungal growth.

2.3 Larval Rearing Protocol

Newly hatched larvae (~28 h post-fertilization) were randomly distributed into rearing tanks according to the designated density treatments. Each tank (20 L) was fitted with a biological sponge filter (XY-2830) and supplied with continuous aeration to ensure adequate oxygenation (DO > 6.5 mg/L). Temperature was maintained at 27 ± 0.5°C, and a moderate light intensity of ~150 lux was provided using overhead LED daylight panels, simulating natural photoperiod conditions.

Feeding commenced at 48 hours post-hatch, beginning with newly hatched *Artemia* nauplii. From day 7, larvae were co-fed with a commercial micro-particulate diet (Otohime B1, Japan), with particle sizes <150 µm and containing 47% crude protein, 10% fat, 1% fiber, and DHA-rich fish oil, ensuring optimal nutrition during the weaning transition. Full weaning was completed by day 14. Feeding occurred three times daily (at 08:00, 14:00, and 20:00), at a rate of 8–10% of body weight per day. A 30% water exchange was performed daily using dechlorinated aged water (24 hours), and debris was removed via gentle siphoning to maintain hygienic conditions and reduce organic load.

2.4 Water Quality Monitoring

Water quality was monitored every three days at 08:00 using calibrated instruments. Temperature and pH were recorded using a HANNA HI98129 combo meter, and dissolved oxygen (DO) was measured using a YSI Pro20 handheld oxygen meter. Total ammonia nitrogen (NH_3) and nitrite (NO_2^-) levels were assessed with API® colorimetric test kits. All readings were compared to optimal larval aquaculture thresholds: temperature (26–28°C), pH (6.8–7.5), DO (>6.0 mg/L), NH_3 (<0.05 mg/L), and NO_2^- (<0.1 mg/L) (Boyd & Tucker, 2012; FAO, 2021). No water quality parameter exceeded the acceptable range during the experiment.

2.5 Biological Performance Measurements

Reproductive and larval performance were evaluated using the following indicators:

- Fecundity (eggs/spawn): Number of fertilized eggs collected per female per spawning event.
- Hatchability (%): (Number of hatched larvae/number of fertilized eggs) × 100.

- Survival Rate (SR, %): (Final number of larvae on day 28 / Initial stocked larvae) × 100.
- Specific Growth Rate (SGR, %/day): SGR = [(ln Wt – ln W0) / t] × 100; where Wt and W0 represent final and initial mean larval weight, respectively, and *t* is the duration in days.
- Feed Conversion Ratio (FCR): Total feed offered (g) / biomass gain (g).
- Color Intensity (ΔE): Fluorescence was quantified using a Konica Minolta CR-400 colorimeter under a D65 standard lighting condition. The ΔE (Delta E) value was calculated using the CIE Lab* system, representing the perceptible difference in color brightness.

All biological data were recorded weekly, and fluorescence assessments were conducted at the end of the 28-day larval phase.

2.6 Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 26. Before conducting statistical tests, Shapiro–Wilk tests were used to assess normality, and Levene's tests were used to verify homogeneity of variances. A two-way ANOVA was employed to examine the main effects of substrate type, stocking density, and their interaction on each response variable. When significant differences ($p < 0.05$) were detected, Duncan's Multiple Range Test (DMRT) was applied for post hoc comparisons. Data are reported as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

3.1 Spawning Performance and Hatchability

The reproductive performance of *Gymnocorymbus ternetzi* (GloFish Tetra) varied significantly across substrate types ($p < 0.05$). The natural aquatic vegetation substrate (S2) resulted in the highest fecundity, averaging 330.2 ± 10.8 eggs per spawning event, followed by the artificial substrate (S1: 294.5 ± 8.7 eggs) and the control group without substrate (S0: 245.6 ± 7.2 eggs). Behavioral observations showed that pairs in the S2 group-initiated courtship activities—such as synchronized swimming and fin fluttering—sooner (average latency: 1.7 ± 0.2 hours) than in the S0 group (3.1 ± 0.4 hours), indicating a substrate-induced enhancement in spawning readiness. Additionally, spawning frequency (number of events per week) was highest in the S2 tanks (3.2 ± 0.3), suggesting that substrate enrichment fosters reproductive success.

Fertilization rates were also influenced by substrate. The S2 group had the highest fertilization rate at 91.5 ± 2.3%, compared to 86.7 ± 3.1% in S1 and 79.2 ± 2.7% in S0. These differences are attributed to the buffering effects of plant interstices, which not only protect gametes from mechanical damage but also stabilize microenvironmental conditions for sperm-egg interactions. In terms of hatchability, S2 yielded the highest value at 82.3 ± 2.7%, significantly surpassing S1 (75.9 ± 2.4%) and S0 (68.2 ± 3.1%). These findings align with previous reports that spawning substrates—particularly complex vegetation—enhance gamete survival by reducing fungal infections and mechanical disturbance (Lash et al., 2020; Vicente et al., 2021).

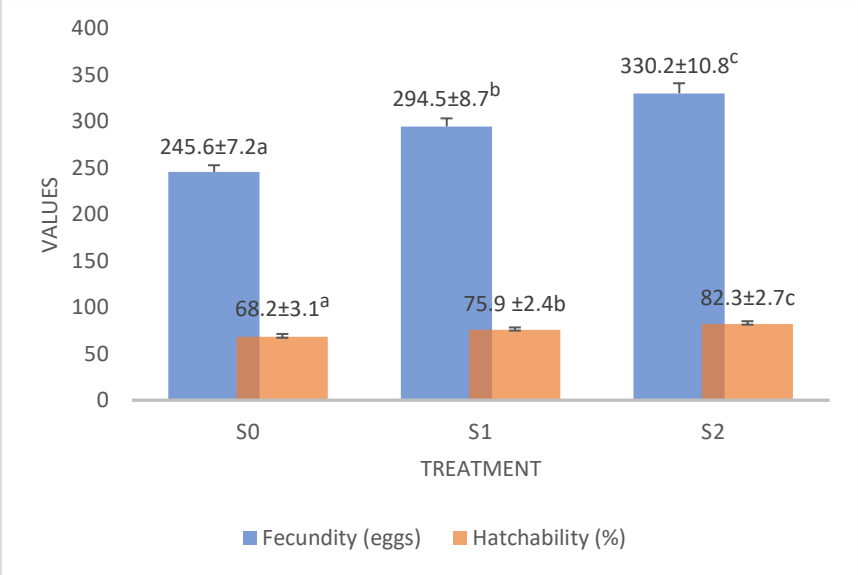

Figure 1. Fecundity and hatchability (%) of GloFish Tetra (Gymnocorymbus ternetzi) under different substrate treatments: no substrate (S0), artificial substrate (S1), and natural aquatic plant substrate (S2). Bars represent means ± standard deviation. Different substrate types showed significant effects on both parameters ($p < 0.05$).

Overall, the use of natural aquatic plants substantially improved reproductive parameters by creating ecologically relevant microhabitats. The lack of substrate in S0 likely induced chronic stress, as evidenced by delayed spawning, lower fecundity, and reduced fertilization. These results underscore the importance of biophysical enrichment in ornamental aquaculture and support the paradigm that mimicking natural spawning cues can improve both productivity and animal welfare (Arechavala-Lopez et al., 2020; Zhang et al., 2022).

3.2 Larval Survival and Growth Performance

Larval performance was significantly influenced by the interaction between substrate type and stocking density ($p < 0.05$). The highest survival rate was observed in the S2-D5 treatment group (81.4 ± 4.2%), while the lowest was in S0-D15 (47.8 ± 3.8%). Mortality analysis revealed that most deaths occurred during the first 7 days post-hatching, a critical period when larvae transition from endogenous to exogenous feeding. In the S0-D15 group, cannibalism and asphyxiation were frequently observed during this stage, highlighting the combined stressors of overcrowding and lack of shelter.

Water quality monitoring across treatments showed slightly elevated ammonia and nitrite levels in high-density tanks (D15), especially in the absence of substrate. In S0-D15, NH_3 levels reached 0.09 mg/L, compared to 0.04 mg/L in S2-D5. Although still within acceptable thresholds (Li et al., 2021), these subtle differences may exacerbate larval stress responses. The presence of *Hydrilla verticillata* likely enhanced nitrification efficiency by providing a surface area for biofilm growth, thereby stabilizing water parameters and improving larval survival.

Specific Growth Rate (SGR) further corroborated these findings. The S2-D5 group attained an SGR of 4.30 ± 0.11%/day, significantly higher than the S0-D15 group (2.96 ± 0.13%/day). Growth variability was also lowest in the S2-D5 group, indicating more consistent development across individuals. The benefits of low stocking density, combined with habitat complexity, include reduced competition,

enhanced access to feed, and lowered basal cortisol levels, all of which contribute to better growth trajectories in ornamental fish (Jia et al., 2022; Swain et al., 2022).

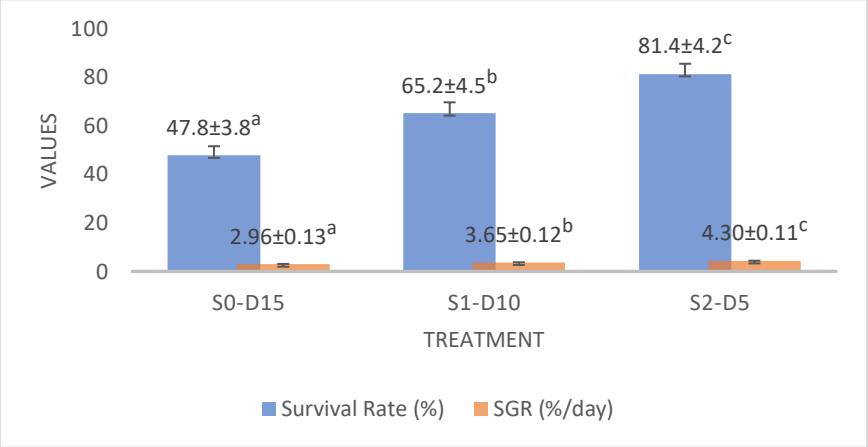

Figure 2. Larval survival rate (%) and specific growth rate (SGR, %/day) of GloFish Tetra under combinations of substrate and stocking density: S0-D15 (no substrate, 15 larvae/L), S1-D10 (artificial substrate, 10 larvae/L), and S2-D5 (natural substrate, 5 larvae/L). Bars represent means ± standard deviation. S2-D5 demonstrated significantly higher survival and growth ($p < 0.05$).

3.3 Feed Utilization and Fluorescent Color Development

Feed Conversion Ratio (FCR) varied significantly between treatments ($p < 0.05$), reflecting differences in metabolic efficiency and feeding hierarchy. The S2-D5 group recorded the lowest FCR at 1.31 ± 0.07, indicating efficient biomass conversion. This can be attributed to the reduced aggression and stable feeding zones provided by the vegetation. In contrast, the S0-D15 group had the highest FCR (1.92 ± 0.10), likely due to erratic feeding patterns, stress-induced anorexia, and increased energy expenditure for territorial defense. These findings support the concept that both abiotic (substrate) and biotic (density) factors influence the energetic efficiency of larval stages (Morimoto et al., 2022; Opare et al., 2022).

Fluorescence intensity, as measured by ΔE values, differed significantly across treatments. The S2-D5 group showed the highest color expression (ΔE = 17.6 ± 1.0), while the S0-D15 group recorded the lowest (ΔE = 12.9 ± 0.9). A ΔE difference of 4.6 units is visually perceptible to the human eye, particularly under blue or ultraviolet lighting, which is commonly used to display GloFish in aquaria. Consumer preference studies suggest that even minor variations in fluorescence can affect marketability, with brighter fish commanding higher prices (Douglas et al., 2021).

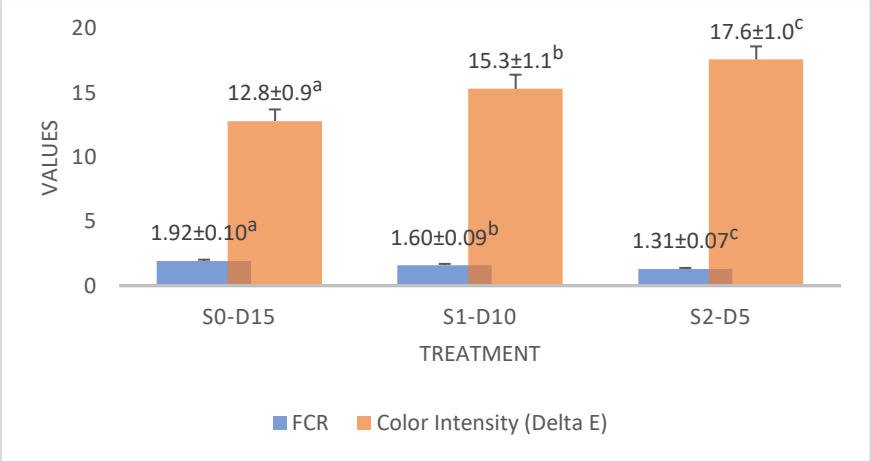

Figure 3. Feed conversion ratio (FCR) and fluorescent color intensity (ΔE) of GloFish Tetra reared under various rearing conditions. Treatments include S0-D15, S1-D10, and S2-D5. The S2-D5 group achieved the most efficient feed utilization and the most vivid fluorescence. Bars represent means ± standard deviation. Differences across treatments were statistically significant ($p < 0.05$).

At the molecular level, GloFish express fluorescent proteins (e.g., GFP, RFP) under the control of muscle-specific promoters. The expression and folding of these proteins are energy-intensive and can be negatively affected by chronic stress, poor nutrition, or oxidative damage. The S2-D5 treatment likely provided optimal physiological conditions—low oxidative stress, good water quality, and adequate nutrition—leading to upregulation of fluorescence pathways. Conversely, the S0-D15 group exhibited signs of reduced pigment deposition and faded hues, likely due to environmental stressors suppressing protein translation or inducing proteolytic degradation.

These findings demonstrate that ornamental quality is inextricably linked to animal welfare. Aesthetic traits such as fluorescence are not merely cosmetic but serve as sensitive indicators of underlying physiological health. By optimizing rearing conditions, hatcheries can enhance both the biological efficiency and commercial value of transgenic ornamental fish.

CONCLUSION

This study demonstrates that breeding and rearing performance of transgenic GloFish Tetra (Gymnocorymbus ternetzi) in tropical freshwater aquaculture is significantly influenced by substrate type and larval stocking density. Natural aquatic vegetation (Hydrilla verticillata) combined with a low stocking density of 5 larvae/L (S2-D5) yielded optimal outcomes across all measured parameters, including fecundity, hatchability, larval survival, specific growth rate, feed conversion ratio, and fluorescent color intensity. The implementation of environmental enrichment and reduced larval crowding not only enhanced biological efficiency but also improved ornamental quality, particularly in terms of fluorescence expression, which is a key determinant of market value. These findings provide an evidence-based framework for developing standardized hatchery protocols tailored to tropical conditions, particularly for smallholder ornamental fish producers. In conclusion, integrating habitat complexity and density regulation provides a scalable and sustainable approach to enhancing the production and aesthetic appeal of transgenic ornamental fish. Future research should explore the long-term genetic and reproductive stability of GloFish strains across

successive generations under variable field conditions to further support commercialization and welfare-based practices in ornamental aquaculture.

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