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Realized gains in survival from divergent selection in Nile tilapia validated under laboratory and field conditions

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Introduction

The intensification of aquaculture production has increased vulnerability to infectious diseases and environmental stressors, making survival at harvest a critical determinant of profitability. Economic analysis from commercial farms in the Philippines indicate that each 1% increase in survival at harvest results in approximately 1.29% higher net profit for tilapia farmers (Joshi *et al*., 2023). To address disease-related losses, a genetically selected *Streptococcus*-resistant Nile tilapia strain was developed and commercially released in 2020, demonstrating 30% higher survival under Streptococcosis outbreaks ($p<0.001$), and successful adoption in the Philippines, Vietnam and Colombia (Delphino *et al*., 2022; Joshi *et al*., 2023).

Building on this progress, the GenoMar breeding program was strategically restructured in 2022 into two parallel breeding lines: (1) a Growth Line, targeting harvest weight and growth rate, and (2) a Robust Line, emphasizing survival and resistance to multiple pathogens. Multi-trait genomic selection was implemented, assigning 60–70% relative weighting to the primary traits of each line. The objective of this study was to compare survival performance between these two lines under both experimental disease challenges and commercial production conditions.

Materials & Methods

Disease challenge test. In generation 3 post-restructuring, controlled laboratory disease challenge trials were conducted to compare resistance between the two lines against key bacterial pathogens affecting Nile tilapia, namely *Streptococcus*, *Flavobacterium*, and *Aeromonas* species. All challenge tests were performed using standardized infection models provided by an external service provider, using fish weighing 10-15 g at the time of challenge. The ethical guidelines followed the protocol of Korea Fishery Research Institute, and approval was obtained from the Welfare Committee of the institution. Challenges with *Streptococcus* and *Flavobacterium* were conducted using cohabitation models involving intraperitoneally injected shedder fish to simulate natural transmission dynamics. In contrast, *Aeromonas* challenges were performed using an immersion challenge model, in which fish were exposed to pathogen-contaminated water, allowing bacterial entry through natural routes such as the skin and gills, thereby closely mimicking field-relevant infection conditions. Each challenge trial was conducted in separate, independent experimental glass tanks with their own Recirculating Aquaculture System (RAS). Pathogen concentrations for each trial were determined based on preliminary dose-finding experiments that resulted in approximately 50% mortality (LD_{50}).

A total of 80 families (70 from the Robust Line and 10 from the Growth Line) were randomly selected for the disease challenge tests. For *Streptococcus* and *Flavobacterium*, six fish per family were intraperitoneally injected with the pathogen and cohabitated with 11 naive fish in experimental tanks to establish the cohabitation infection model. For Aeromonas, 11 fish per family were subjected to immersion exposure and subsequently transferred to holding tanks. Post-infection mortality was recorded every 3 hours for up to 27 days following challenge (or until no consecutive mortality for subsequent 3 days). Fin clips from deceased fish were

collected immediately after mortality was observed. At the end of the experiment, fin clips were also collected from all surviving fish.
As individual fish were not physically tagged, parentage assignment was performed using genomic DNA extracted from fin clip samples to assign the fish to the breeding lines. Genotyping was conducted using the Onil50 SNP array (Joshi *et al*., 2018), comprising 58,466 single nucleotide polymorphisms (SNPs). Survival probabilities associated with each breeding line and pathogen were estimated using Kaplan–Meier survival curves (Kaplan & Meier, 1958), treating surviving fish as censored observations. Kaplan–Meier estimators were calculated using the survfit function in the R package survminer (Kassambara *et al*., 2017), given by

$$\hat{S}(t) = \prod_{t_i \le t} \left(1 - \frac{d_i}{n_i}\right) \quad (1)$$

where, n_i is the number of fish at risk at time t_i, and d_i is the number of fish that died at that time. The differences in survival distributions between breeding lines were evaluated using the non-parametric log-rank test. Relative percent survival (RPS) was calculated for each pathogen challenge by comparing the cumulative mortality of the Robust line to that of the Growth line at the end of the challenge period.

Field trial under high disease and environmental stress conditions. By generation 6 post-restructuring, common-garden field trials were conducted under commercial pond and cage production systems in the Philippines to validate the laboratory results under high disease pressure and environmental stress conditions. Standard commercial management practices, including stocking densities and feeding regimes (BFAR, 2022), were applied throughout the trials. Experimental fish were produced by spawning 77 families from the Growth line and 80 families from the Robust line, with family spawning conducted through four weekly collections for each line. As individual fish required tagging, families were initially reared separately in 1 × 1 m hapas in a same pond until they reached tagging size, with 100 fry per family stocked per hapa. Fish were PIT-tagged at an average body weight of approximately 20 g, allowed a one-week recovery period, and then transferred to either cage or pond production systems.
A total of 2,389 fish (1,294 from 77 families of the Growth line and 1,095 from 80 families of the Robust line) were stocked in cages of 5mx5mx8m and 1,573 fish (495 from the Growth line and 1,078 from the Robust line) were stocked in ponds of 300 m². The uneven numbers of fish between breeding lines reflected differences in family-level survival prior to stocking. The pond site was located in an area characterized by high disease pressure and elevated salinity. The cage site was located in a reservoir with high disease pressure and low water temperatures, and the culture period occurred during the typhoon season, during which the cages remained intact. Feeding was based on BFAR guidelines (BFAR, 2000) using commercially available feed. Final harvest was conducted after 60 days of grow-out in ponds (average harvest weight 228 g) and 70 days of grow-out in cages (average harvest weight 322 g). Individual PIT tag and the weight of the fish were collected during harvest for all the surviving fish. RPS was calculated for each production system by comparing the cumulative mortality of the Robust line to that of the Growth line at the harvest.

Results

In the laboratory disease challenge test, mortality patterns differed among the three bacterial pathogens, with the Robust line consistently showing higher survival than the Growth line (Fig. 1(a)). For Streptococcus, mortality in the Growth line started on day 1, whereas in the Robust line it began on day 2, with no mortalities observed after day 19 and day 20, respectively. Final cumulative mortalities were 67.26% in the Growth line and 52.71%, in the Robust Line,

yielding an RPS of 21.7% in favor of the Robust line. In the Flavobacterium challenge, mortality began at day 0 in both lines but progressed more slowly in the Robust line, with no mortalities after day 25; cumulative mortalities were 55.83% for the Growth line and 49.12% for the Robust line, corresponding to an RPS of approximately 12%. For Aeromonas, mortality also started at day 0 in both lines, increasing sharply in the Growth line from day 8 and plateauing around day 22, while the Robust line accumulated mortality gradually until day 27. Final cumulative mortalities were 62% in the Growth Line and 53.9% in the Robust Line, giving an RPS of 13.1% in favor of the Robust line.
Kaplan–Meier survival analyses demonstrated significant differences between lines for Streptococcus ($p < 0.001$) and Aeromonas pathogens ($p < 0.05$), confirming consistently higher survival in the Robust line throughout the challenge periods, while differences for Flavobacterium were not statistically significant.

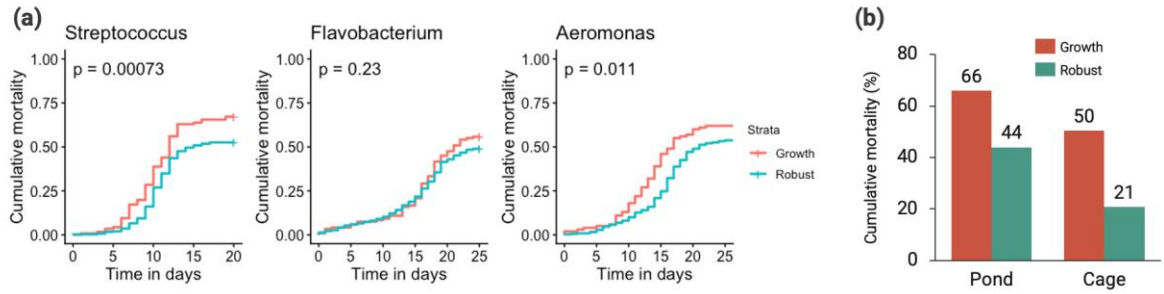

Figure 1. (a) Kaplan–Meier survival curves of Growth and Robust Nile tilapia lines under three different bacterial challenge tests in laboratory. (b) Cumulative mortality for Growth and Robust lines in commercial field trials under pond and cage production systems.

In the field trial (Fig. 1(b)), survival differences between the Growth and Robust lines were consistent with laboratory results. In the pond production system, mortality was 65.86% in the Growth line compared to 43.97% in the Robust line, corresponding to an RPS of 33.23% in favor of the Robust line. In the cage system, mortality was 50.31% in the Growth line versus 20.73% in the Robust line, yielding an RPS of 58.8%. These results indicate that the Robust line maintained higher survival under commercial conditions with high disease pressure and environmental stress.

Discussion

Divergent multi-trait genomic selection successfully produced two Nile tilapia lines with distinct survival profiles. The Robust line consistently outperformed the Growth line across multiple pathogens and production systems, confirming that prioritizing survival and disease resistance in the selection index leads to meaningful gains in harvest survival. The use of immersion and cohabitation challenge models strengthened the relevance of laboratory results by closely reflecting natural infection routes commonly observed in commercial aquaculture (Nguyen *et al*., 2002).
Despite evaluations being conducted in different generations, RPS values ranging from 12% to 59% across challenges and field trials demonstrate stable and transferable genetic gains in survival. RPS was used as the primary measure of genetic gain because it provides a direct, biologically interpretable comparison of survival outcomes between breeding lines, whereas genetic standard deviation units are difficult to translate into expected improvements for survival traits.

Conclusions

This study provides clear evidence that selection for robustness substantially improves survival at harvest in Nile tilapia under both experimental and commercial conditions. Genetic improvement for disease resistance represents a practical and effective strategy to enhance resilience, reduce losses, and increase profitability in tilapia aquaculture. Maintaining separate Growth and Robust lines allows producers to select strains aligned with specific production goals, offering flexibility while maximizing overall industry sustainability.

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