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PENSOFT

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Development of a saline-tolerant YY supermale UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*) (Actinopterygii, Cichliformes, Cichlidae) using molecular marker-assisted selection

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Abstract

The tilapia aquaculture industry faces increasing challenges from saline intrusion into freshwater systems and consumer concerns over hormone-treated fish. This study developed a molecular marker-assisted YY male technology in the saline-tolerant UPV SpiN tilapia hybrid {*Oreochromis spilurus* (Günther, 1894) × *Oreochromis niloticus* (Linnaeus, 1758)} to enable all-male progeny production without direct hormone treatment. A primer set (UPV-SpiN-M5) targeting the *amhy* gene, a key determinant of male sex differentiation, was utilized. Crosses between neofemales (sex-reversed XY females; XY ♀) and natural XY males produced YY supermales, and mating these with natural XX females yielded 100% genotypically male fingerlings all exhibiting the Y-specific band (~523 bp). Despite the limited number of successful spawns among YY supermale × XX female crosses, the successful families produced viable all-male fry, demonstrating the effectiveness of the molecular-assisted approach. These results demonstrate the feasibility of hormone-reduced monosex production in saline-tolerant tilapia and provide a foundation for scalable, sustainable seed production systems suitable for both freshwater and brackish water aquaculture. Future studies should focus on refining broodstock management to improve spawning success, validate genotypic and phenotypic sex at maturity, assess growth and economic performance of YY-derived males, and explore the use of pseudofemales (sex-reversed YY females; YY ♀) to enable the mass production of YY supermales and enhance overall production efficiency.

Keywords

all-male tilapia production, amhy gene, brackish water aquaculture, genotyping, hormone-reduced monosex production, YY male technology

* These authors contributed equally to this work.

Introduction

Tilapia ranks among the most widely farmed finfish species globally, with production reaching over three million tonnes in 2010 (FAO 2013). Since then, production has continued to increase, exceeding five million tonnes in 2022 (FAO 2024). This growth is driven by rising demand in both domestic and international markets. Currently, tilapia is cultured in more than 100 countries (BFAR 2022; El-Sayed and Fitzsimmons 2023). Among the various farmed species, Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) remains the most widely cultivated due to its rapid growth, adaptability to diverse environmental conditions, and favorable culture traits (El-Sayed and Fitzsimmons 2023).

However, a persistent challenge in tilapia aquaculture is early sexual maturation during the grow-out phase. Early maturation leads to uncontrolled spawning, producing fry and juveniles within production systems (Beardmore et al. 2001). This results in overpopulation, competition for food and space, and a wide size variation at harvest, ultimately reducing production efficiency and economic returns (Mair et al. 1997). To mitigate these issues, all-male culture systems have been widely adopted to ensure uniform growth and maximize yield (El-Sayed 2006). Traditionally, this is achieved through the administration of sex steroids such as 17α-methyltestosterone (MT) during early fry stages to induce masculinization (Beardmore et al. 2001).

In the Philippines, tilapia is the second most cultured finfish and the most commonly consumed fish among the local population (BFAR 2024). However, national production has gradually declined in recent years due to various interlinked environmental and socioeconomic factors. According to Guerrero (2019), stagnation in production is attributed to the limited expansion of freshwater farming areas, declining water quality, and increasing vulnerability to climate-related events. As an archipelagic nation, about 93% of tilapia farming in the Philippines are still being cultured in freshwater systems (BFAR 2022). Additionally, saline intrusion, exacerbated by sea-level rise and coastal groundwater salinization, has emerged as a major threat, causing physiological stress and mass mortalities in affected freshwater culture systems (Dubey et al. 2017; PCAARRD 2024). In parallel, market concerns over the use of synthetic hormones in aquaculture have intensified both locally and globally (Hoga et al. 2018). Although various studies have shown that hormone residues in fish tissues and surrounding water following hormonal treatments are often below detection limits or show no significant differences between treated and untreated groups (Hoga et al. 2018), consumer perceptions continue to influence the marketability and acceptance of hormone-treated seafood products (Fegan and Fitzsimmons 2008; Leng et al. 2020).

The saline-tolerant UPV SpiN tilapia hybrid {*Oreochromis spilurus* (Günther, 1894) × *O. niloticus*} (Fig. 1) has been established as a candidate for genetic improvement programs because it combines salinity tolerance with rapid growth (Fernandez 2003). Dinaga et al. (2025) advanced this work by producing neofemales (sex-reversed XY females; XY ♀) through 17β-estradiol (E2) treatment and confirming their genotype using *amhy*-based PCR. However, their potential to generate YY progeny remained untested. Building on that study, the present work aimed to evaluate whether confirmed neofemales, when crossed with genotypically verified natural XY males, can produce viable YY supermales. This approach enables the mass production of genetically all-male offspring without applying steroid hormones directly to fry, since hormonal intervention is restricted to the feminization phase during YY broodstock development (Mair et al. 1997). The use of molecular marker-assisted selection further strengthens this process by allowing rapid and accurate identification of YY supermales (Sun et al. 2014; Jiang et al. 2022). Sex-linked genetic markers provide efficient screening of sex genotypes, reducing broodstock development time and improving breeding success (Lee et al. 2003; Palaiokostas et al. 2013; Jiang et al. 2022). These putative YY supermales were subsequently paired with natural XX females to test their ability to produce genetically all-male offspring.

This study represents a critical step toward establishing a hormone-reduced, genetically based system for mass-producing all-male saline-tolerant UPV SpiN tilapia, offering a scalable and sustainable strategy to enhance aquaculture productivity.

Materials and methods

Broodstock preparation and genotypic identification. Phenotypic sex sorting, hormonal feminization, and genotypic identification of neofemales (XY ♀) from F1 generation were conducted previously by Dinaga et al. (2025). Briefly, UPV SpiN-tilapia fry were feminized using 17β-estradiol (E2), and genotypic sex was determined using the Marker-5 primer set (Jiang et al. 2022), which targets the *amhy* gene located on linkage group 23 (LG23). This allowed for the identification of phenotypic females possessing XY chromosomes. In addition to the Marker-5 primer, a new primer set, UPV-SpiN-M5, was designed by the project to target the same *amhy* gene. The design was adapted from the Marker-5 primer set by Jiang et al. (2022). Both markers produced consistent sex-specific amplification patterns when tested on the same DNA samples of natural XX females and neofemales, confirming the diagnostic accuracy of the UPV-SpiN-M5 primer for genotypic sex identification in the UPV SpiN hybrid. As shown in Fig. 2, UPV-SpiN-M5 primer amplifies fragments of approximately 963 bp from the X chromosome and 523 bp from the Y chromosome (forward: 5′–[GCTCCCGAACAGTTTCTTTTCCAC]; reverse: 5′–[CCTCCTCTTCTTCTTCGTGTCCAC]).

Figure 1. Representative photograph of an adult UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*).

Breeding trial of the F2 generation with YY supermale individuals. A total of 24 confirmed neofemales (mean body weight [MBW]: 200–250 g) and six natural XY males (MBW: 250–300 g) were used for the F2 breeding trial. Each 1-metric ton spawning tank was stocked with one XY male and four neofemales, all genotypically verified using the UPV-SpiN-M5 primer set. The breeders maintained at the salinity of 15‰–20‰ were then conditioned separately to freshwater (0‰) for 14 days. Breeding tanks were maintained at 0‰salinity and monitored daily for the presence of swim-up fry beginning 14 days post-pairing, with collections conducted after 28 days.

A total of 879 swim-up fry was collected from all six families and initially pooled in a 155 L tank. The fry were then randomly distributed into six 1-metric ton tanks under standard hatchery conditions {temperature: 28.20 ± 0.50°C; dissolved oxygen (DO): 6.10 ± 0.10 mg · L^{-1}; ammonia: 0.05 ± 0.02 mg · L^{-1}; all values are presented as mean ± standard deviation (SD)}. After one day of acclimatization, fry were fed a commercial feed (≥45% crude protein, ≥ 10% crude fat, ≤ 5% crude fiber, ≤ 16% crude ash, ≤ 12% moisture, 0.25 mm particle size) and reared under standard hatchery conditions with daily maintenance and partial water changes. Salinity was gradually increased at a rate of approximately 2‰ per day by adding seawater until the desired salinity for each day was reached. Salinity was monitored daily using a handheld refractometer and maintained between 15‰–20‰ thereafter.

Fry were fed ad libitum six times daily from 0 to 7 days post collection (dpc) of swim-up fry. From 8 to 14 dpc, feeding was adjusted to 20% of MBW administered six times daily. From 15 to 28 dpc, feeding was reduced to 15% MBW. From 29 to 90 dpc, fry were fed a commercial diet (≥39% crude protein, ≥ 7% crude fat, ≤ 5% crude fiber, ≤ 12% crude ash, ≤ 12% moisture, ≥ 1.50 mm particle size) at 10% MBW. Feeding was further reduced to around 5% MBW from 91 to 120 dpc using a commercial diet (≥31% crude protein, ≥ 7% crude fat, ≤ 6% crude fiber, ≤ 12% crude ash, ≤ 12% moisture).

After 120 days, fish weighing around 50 g were transferred to pond systems to support further growth. Fish were fed a commercial diet (≥ 31% crude protein, ≥ 7% crude fat, ≤ 6% crude fiber, ≤ 12% crude ash, ≤ 12% moisture), with feeding rates adjusted based on MBW following general tilapia feeding recommendations. Since growth performance was not evaluated in this study, feeding rates were not strictly observed but served as a guide to support normal growth until fish reached 100–150 g, when phenotypic sex sorting and genotyping were conducted.

Phenotypic sex sorting and genotyping of the F2 generation with YY supermales. A total of 471 randomly selected adult UPV SpiN tilapia hybrid were subjected to phenotypic sex sorting and subsequent genotyping. Fin tissue samples (25–30 mg) were excised from the soft dorsal rays following the protocol of Dinaga et al. (2025). A fin excision coding scheme was also applied to enable temporary fish identification (Dinaga et al. 2025). Genomic DNA was extracted using a modified Wizard® Genomic DNA Purification Kit (Promega). Briefly, fin tissues were digested overnight at 65°C in a lysis buffer containing Proteinase K. The lysates were then treated with RNase A, followed by protein precipitation and DNA isolation using isopropanol.

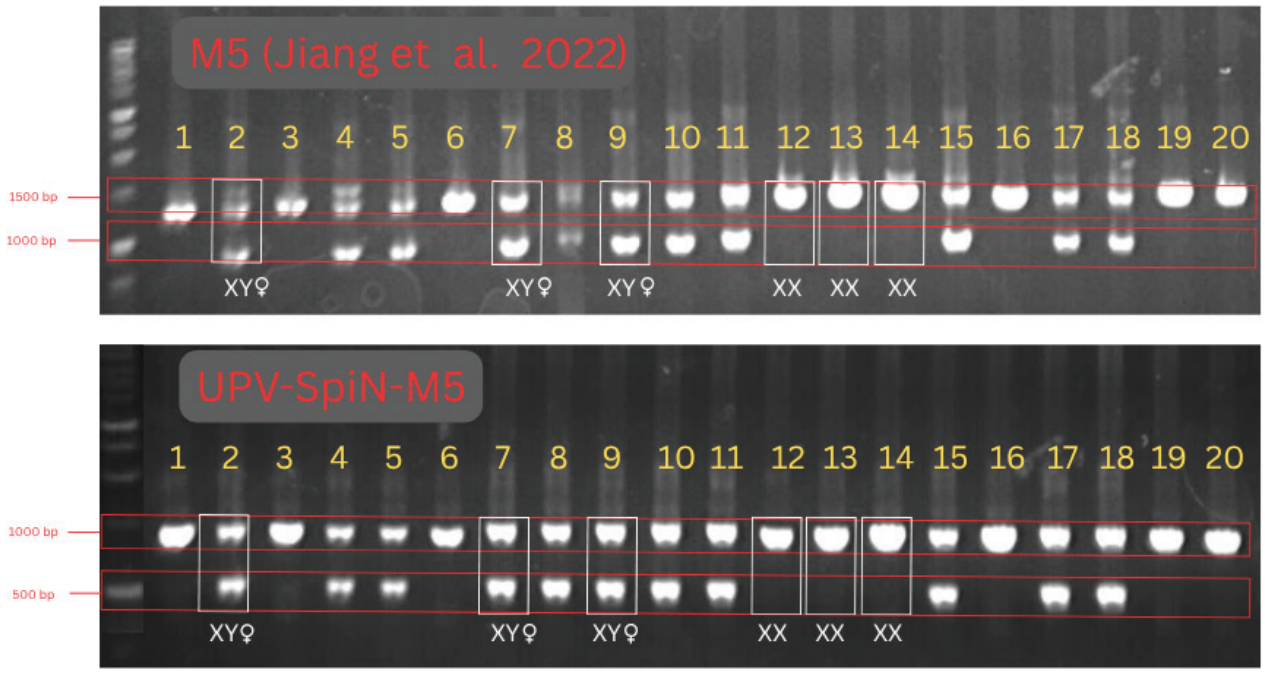

Figure 2. Representative agarose gel electrophoresis showing the amplification profiles of the *amhy* gene from the same DNA samples of natural XX females and neofemales (XY ♀) using the **Marker-5 (Jiang et al. 2022)** and UPV-SpiN-M5 primer sets in the UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*). The **UPV-SpiN-M5** primer set amplified fragments of approximately 963 bp (X-specific) and 523 bp (Y-specific). Both primer sets yielded consistent sex-specific amplification patterns, confirming the reliability of the UPV-SpiN-M5 marker for genotypic sex identification.

DNA pellets were washed with 70% ethanol, air-dried, and rehydrated in nuclease-free water. The purity and concentration of extracted DNA were measured using a NanoDrop spectrophotometer, with acceptable 260/280 absorbance ratios between 1.8 and 2.0. DNA samples were stored at –20°C until further analysis.

For genotyping, a 10 µL PCR reaction mixture was prepared, consisting of 1 µL DNA template (300 ng · mL^{-1}), 5 µL GoTaq® Colorless Master Mix, 0.5 µL each of forward and reverse primers, and 3 µL nuclease-free water. PCR conditions included an initial denaturation at 94°C for 3 min; followed by 34 cycles of denaturation (94°C, 30 s), annealing (53.8°C, 30 s), and extension (72°C, 1.5 min); and a final extension at 72°C for 10 min. PCR products were separated using 1% agarose gel electrophoresis, stained with GelRed, and visualized under a UV transilluminator. The UPV-SpiN-M5 primer set was used to determine the genotypic sex of the progeny.

Breeding trials and genotypic validation of the F3 generation from XX female × YY supermale. Each confirmed YY supermale breeder was paired with three genotypically verified natural XX females in separate 1-metric ton breeding tanks for one family. Three families per breeding batch were established, with spawning conducted across three independent batches at different time points (December 2024, March 2025, and May 2025). Breeding trials followed a 14-day conditioning period prior to pairing. Swim-up fry were monitored starting 14 days post-pairing and continued until 35–42 days. A total of 27 XX females (MBW: 100–150 g) and nine YY supermales (MBW: 300–500 g) were used in the breeding trials comprising of nine families. Breeding tanks were maintained under standard hatchery conditions, and swim-up fry were collected for subsequent genotypic validation.

For the first breeding batch, collected swim-up fry were reared until reaching approximately 5 g. A total of 102 fingerlings were randomly selected, and dorsal fin tissue was excised for chromosomal sex determination. For the second and third breeding batches, 100 fry (10–15 dpc) per batch were randomly selected and sacrificed for genotyping. The posterior portion of each fry, excluding the head and gut, was processed for genomic DNA extraction following the same protocol described above.

ARRIVE 2.0 compliance. This study adhered to the ARRIVE 2.0 (Animal Research: Reporting of in vivo Experiments) guidelines to ensure transparency, reproducibility, and completeness in reporting animal research methodologies (Percie du Sert et al. 2020).

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Results

Genotypic validation of F2 generation from neofemale (XY ♀) × XY male. Genotypic validation of the progeny from confirmed neofemales crossed with XY males is summarized in Table 1. The percentage distribution of XX, XY, and YY individuals was calculated based on the total number of samples analyzed. These observed genotypes are consistent with the expected outcomes based on a Punnett square for an XY × XY cross, which predicts a 1:2:1 ratio of XX, XY, and YY genotypes, respectively. Representative gel electrophoresis results showing the diagnostic band patterns for each genotype (XX, XY, and YY) are presented in Fig. 3, confirming the successful amplification and differentiation using the UPV-SpiN-M5 primer set.

Table 1. Percent population of genotypically determined progeny from confirmed neofemales (sex-reversed XY females, XY ♀) and natural XY males of UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*). A total of 471 randomly sampled mature individuals were analyzed for genotypic sex identification.

Genotype	No. of samples	Population [%]
YY	117	24.84
XY	236	50.11
XX	118	25.05
Total	471	100

Genotypic validation of F3 generation from XX female × YY supermale. Of the three families established in each breeding batch, only one family successfully spawned and produced swim-up fry. This pattern was consistent across all three breeding batches (December 2024, March 2025, and May 2025). As shown in Table 2, a total of 721 swim-up fry were produced across three breeding batches, with 302 individuals randomly selected for genotyping. All genotyped samples consistently exhibited the Y-specific *amhy* band (~523 bp) when amplified with the UPV-SpiN-M5 primer set, yielding a 100% detection rate of Y chromosomes. The representative gel electrophoresis further validates the presence of the Y-specific marker across samples, providing strong molecular evidence that the F3 generation maintained the intended all-male genotype (Fig. 4). A few samples that failed to amplify either X- or Y-specific bands were excluded due to poor DNA quality, and replacement samples were analyzed to maintain 100–102 genotyped individuals per batch.

Discussion

The presently reported study successfully developed UPV SpiN YY supermales that are tolerant to saline environments, with the aid of molecular sex markers. While breeding and early larval development were usually conducted under freshwater conditions for this hybrid, the resulting fry demonstrated tolerance to elevated salinities, as also reported in previous studies, making them suitable for brackish water aquaculture (Intoy and Traifalgar 2021; Huervana et al. 2022; Dinaga et al. 2025). In the Philippines, previous efforts to develop YY male technology focused on freshwater *O. niloticus* (see Abella et al. 2001). However, these conventional breeding approaches typically required hundreds of breeding pairs and involved laborious progeny testing to confirm YY genotypes (Mair et al. 1997), making the process inefficient and difficult to scale.

In contrast, the molecular marker-assisted approach adopted in this study has significantly improved the efficiency and productivity of YY supermale production. By using sex-specific markers targeting the X and Y chromosomes, genotypic sex could be rapidly and accurately determined at early stages, thereby reducing the need for extensive test-crossing and phenotypic validation (Sun et al. 2014; Jiang et al. 2022). This streamlined approach greatly shortens the breeding cycle and facilitates the establishment of YY broodstock lines.

Currently, monosex tilapia production in the aquaculture industry commonly involves the use of synthetic androgens such as MT, for sex reversal during early fry stages (Beardmore et al. 2001). However, growing concerns among consumers and regulatory agencies about hormone-treated fish have led to a push for more natural and residue-free alternatives. In the present study, E2, a naturally occurring estrogen, was used for feminization during YY supermale development. E2 is generally considered

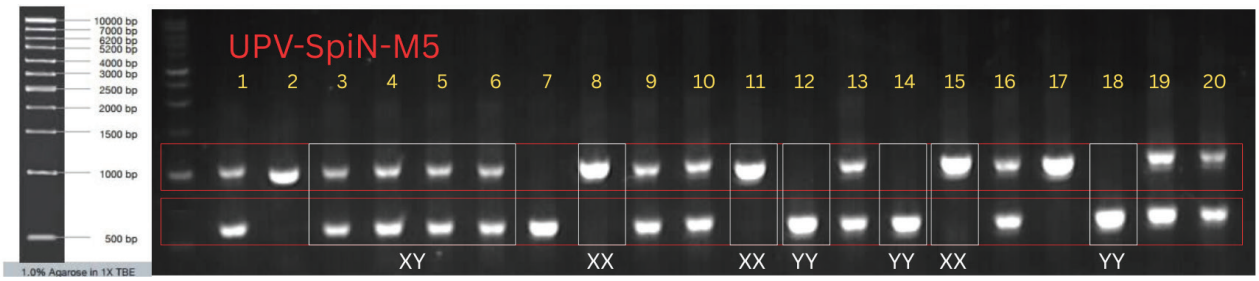

Figure 3. Representative gel electrophoresis profiles of UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*) showing diagnostic banding patterns for XX, XY, and YY genotypes. Amplification with the **UPV-SpiN-M5** primer set yielded distinct bands at 963 bp (X-specific) and 523 bp (Y-specific), allowing clear differentiation of sex genotypes.

Table 2. UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*) swim-up fry collected and genotyped across three breeding batches (December 2024, March 2025, and May 2025). Each batch consisted of three families (3 natural XX females: 1 YY supermale per family). The table presents the total number of swim-up fry, the number of samples analyzed, and the proportion carrying the Y-specific *amhy* band.

Breeding batch #	Family #	Total no. of swim-up fry collected	No. of randomly selected fingerlings genotyped	With Y-specific band [%]
	1	316		
1	2	0	102	100
	3	0		
	4	0		
2	5	147	100	100
	6	0		
	7	258		
3	8	0	100	100
	9	0		
Total		721	302	100

safer and more acceptable than synthetic hormones, both due to its endogenous nature and the evidence indicating its rapid degradation in fish tissues within hours to a few days (Piferrer 2001; Specker and Chandlee 2003; Hoga et al. 2018). Nevertheless, future studies quantifying hormone residues in fry and market-size UPV SpiN tilapia are recommended to support claims of food safety and improve consumer trust.

While E2 provided a safer and more natural feminization approach, challenges were observed during subsequent breeding, where spawning success remained inconsistent. The low spawning success observed, with only one out of three groups producing swim-up fry per breeding batch, suggests the presence of significant reproductive or behavioral constraints affecting the reliability of seed production in this system. This pattern, repeated across all three batches, may reflect challenges such as suboptimal broodstock compatibility, environmental stressors, or unexpected variation in reproductive readiness and pairing dynamics of the selected fish. Although the successful groups produced a total of 721 viable fry, the limited number of successful spawns underscores the need to refine broodstock management, optimize pairing ratios, and standardize environmental conditions to enhance spawning consistency and fry yield. Addressing this bottleneck will be critical for scaling up YY-based production in commercial hatcheries.

Despite the limited number of successful spawns, the quality of offspring produced was remarkable. One of the key findings of this study is the 100% efficiency in producing genetically all-male offspring from UPV SpiN YY supermales, as demonstrated by the consistent amplification of Y-specific bands across all samples. According to Punnett square principles, crosses between YY supermales and XX females should yield only XY progeny, and the uniform detection of Y-specific bands confirms this theoretical expectation. While most individuals exhibited both X- and Y-specific bands, a subset displayed only the Y-specific band. This does not imply the presence of YY individuals, which are genetically impossible from a YY × XX cross, but is more likely due to differences in amplification efficiency, suboptimal DNA template quality, or preferential amplification of smaller fragments in multiplex PCR reactions (Deng et. al. 2000; Elnifro et al. 2000).

Recent studies have demonstrated that YY male technology can indeed produce 100% male progeny under controlled conditions. Rahman and Sarder (2010) documented that YY supermales paired with XX females produced 83%–100% male offspring in five families, while Liu et al. (2025) found that four out of five YY × XX families yielded 100% male progeny. Nonetheless, it is well established that not all XY individuals necessarily develop as phenotypic males, as sex differentiation in tilapia can be influenced by complex interactions among genetic, environmental, and epigenetic factors (Mair et al. 1997; Baroiller et al. 2009; Triay et al. 2022; Liu et al. 2025). It is therefore important to note that the present findings are based solely on genotypic identification of fry. Moreover, the study was limited to a few successfully spawning families; hence, validation across a larger number of families in future trials is necessary to strengthen the robustness and applicability of the UPV SpiN YY male technology. Future studies should include concurrent phenotypic validation at maturity. Nevertheless, the observed 100% genotypic efficiency surpasses that of MT-induced sex reversal, which typically achieves 68%–99% male offspring (Kohinoor et al. 2003; Shamsuddin et al. 2012).

Beyond efficiency, this finding highlights the practical potential of YY technology as a hormone-reduced alternative for reliable monosex seed production, particularly in saline-tolerant hybrids suited to brackish water aquaculture. Previous studies in *O. niloticus* (see Islam et al. 2015; Opiyo et al. 2020) have shown that genetically all-male populations often exhibit superior growth and uniformity. To fully establish the commercial viability of this approach, comparative trials evaluating the growth performance, survival, and feed conversion efficiency of all-male UPV SpiN tilapia hybrid offspring produced via YY male technology against hormone-treated or mixed-sex cultures are recommended. In addition, a comprehensive economic analysis will be essential to assess the cost-effectiveness of adopting this breeding strategy at hatchery and farm levels.

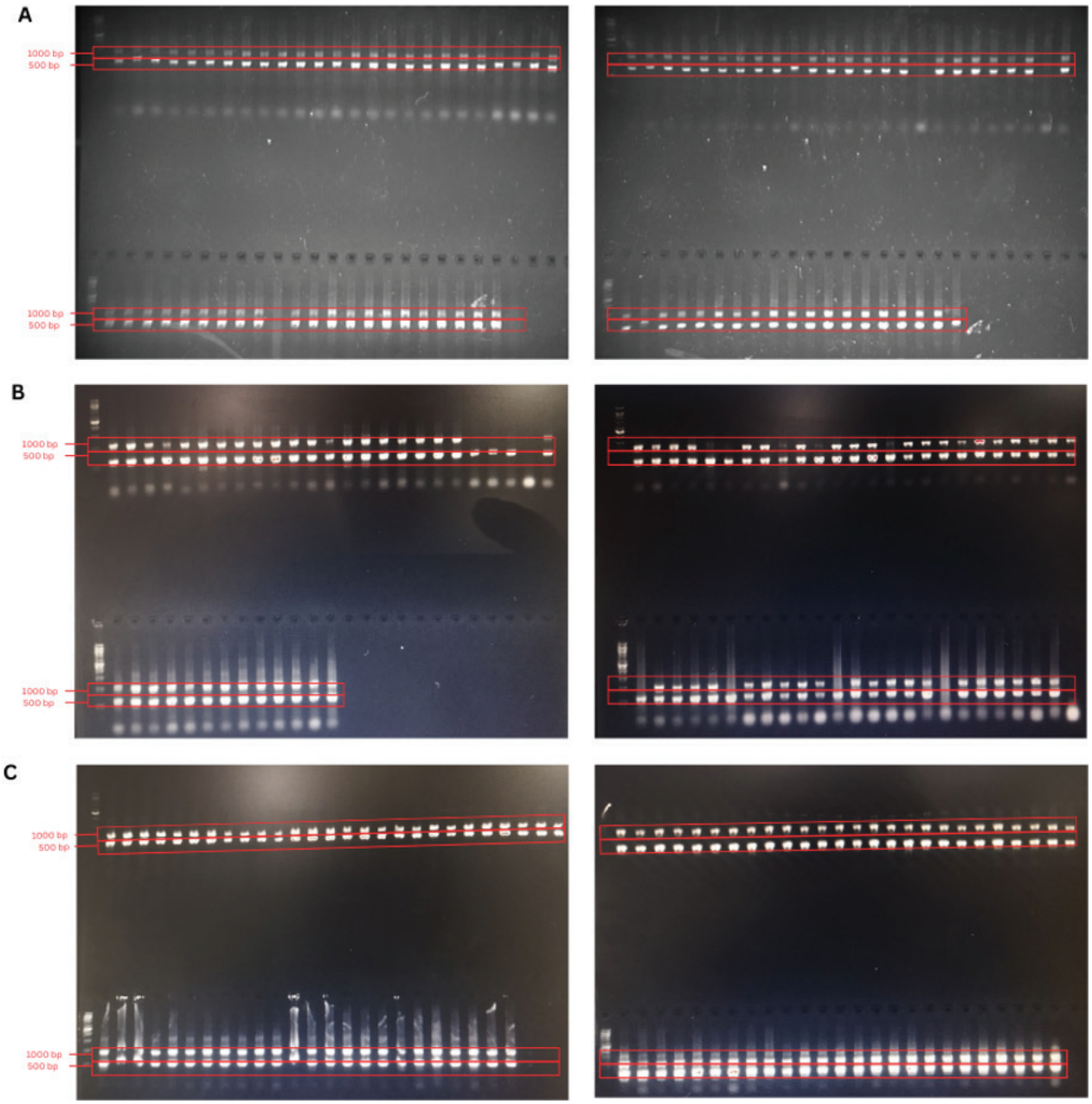

Figure 4. Representative gel electrophoresis results of PCR-based genotyping of fingerlings and fry samples from UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*) produced by YY supermale and natural XX female parents. (**A**) Batch 1 fingerling samples processed within 19–26 February 2025. (**B**) Batch 2 fry samples processed within 2–23 May 2025. (**C**) Batch 3 fry samples processed within 30 June–8 July 2025. A 1 kb DNA ladder was used as a molecular size reference. All samples were amplified using the UPV-SpiN-M5 primer set.

Integrating YY male technology into brackish water hatchery systems could provide a sustainable solution to the growing demand for tilapia seedstock in coastal and estuarine areas, where saline intrusion increasingly threatens traditional freshwater farms. By combining genetic approaches with adaptive hatchery protocols, the UPV SpiN tilapia hybrid has the potential to serve as a model breed for resilient, hormone-reduced aquaculture. Such innovations not only address environmental and consumer concerns but also contribute to long-term food security and industry competitiveness in the Philippines and other regions facing similar challenges.

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References

Abella TA, Abucay JS, Sevilleja RC, Mair GC (2001) The development and commercialization of the YY-male tilapia technology. Transactions of the National Academy of Science and Technology, Philippines 23(2): 71–77. https://doi.org/10.57043/transnastphl.2001.5115

Baroiller JF, D'Cotta H, Bezault E, Wessels S, Hoerstgen-Schwark G (2009) Tilapia sex determination: Where temperature and genetics meet. Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology 153: 30–38. https://doi.org/10.1016/j.cbpa.2008.11.018

Beardmore JA, Mair GC, Lewis RI (2001) Monosex male production in finfish as exemplified by tilapia: Applications, problems, and prospects. Aquaculture (Amsterdam, Netherlands) 197(1–4): 283–301. https://doi.org/10.1016/S0044-8486(01)00590-7

BFAR [Bureau of Fisheries and Aquatic Resources] (2022) Tilapia industry roadmap 2022–2025. Department of Agriculture – Bureau of Fisheries and Aquatic Resources, Quezon City, Philippines. https://www.bfar.da.gov.ph/wp-content/uploads/2022/11/Tilapia-Industry-Roadmap-1.pdf

BFAR [Bureau of Fisheries and Aquatic Resources] (2024) 2023 Philippine fisheries profile. Department of Agriculture – Bureau of Fisheries and Aquatic Resources, Quezon City, Philippines. https://www.bfar.da.gov.ph/wp-content/uploads/2025/02/2023-Philippine-Fisheries-Profile.pdf

Deng HW, Zhou Y, Recker RR, Johnson ML, Li J (2000) Fragment size difference between multiplex and singleplex PCR products and their practical implications. BioTechniques 29(2): 298–308. https://doi.org/10.2144/00292st05

Dinaga CJP, Genciana ABN, Failaman AA, Fontanilla MAG, Estrada RA, Mameloco EJG, Ferriols VMEN, Traifalgar RFM, Lumayno SDP (2025) Feminization of a saline-tolerant tilapia hybrid, UPV SpiN (*Oreochromis spilurus* × *O. niloticus*) (Actinopterygii, Cichliformes, Cichlidae) using 17β-Estradiol: Effects of dose and administration method. Acta Ichthyologica et Piscatoria 55: 227–235. https://doi.org/10.3897/aiep.55.161420

Dubey SK, Trivedi RK, Chand BK, Mandal B, Rout SK (2017) Farmers' perceptions of climate change, impacts on freshwater aquaculture and adaptation strategies in climatic change hotspots: A case of the Indian Sundarban delta. Environmental Development 21: 38–51. https://doi.org/10.1016/j.envdev.2016.12.002

El-Sayed AFM (2006) Tilapia culture. CABI Publishing, Wallingford. https://doi.org/10.1079/9780851990149.0000

El-Sayed AFM, Fitzsimmons K (2023) From Africa to the world—The journey of Nile tilapia. Reviews in Aquaculture 15(Suppl. 1): 6–21. https://doi.org/10.1111/raq.12738

Elnifro EM, Ashshi AM, Cooper RJ, Klapper PE (2000) Multiplex PCR: Optimization and application in diagnostic virology. Clinical Microbiology Reviews 13(4): 559–570. https://doi.org/10.1128/CMR.13.4.559

FAO [Food and Agriculture Organization] (2013) The state of world fisheries and aquaculture 2012. Food and Agriculture Organization of the United Nations, Rome.

FAO [Food and Agriculture Organization] (2024) The state of world fisheries and aquaculture 2024: Blue transformation in action. Food and Agriculture Organization of the United Nations, Rome. https://doi.org/10.4060/cd0683en

Fegan D, Fitzsimmons K (2008) Expanding tilapia supply meets growing global market. Global Seafood Alliance Advocate.

Fernandez RA (2003) Saline-tolerant tilapia being developed. Philstar Global.

Guerrero RD III (2019) Farmed tilapia production in the Philippines is declining: What has happened and what can be done. Philippine Journal of Science 148(2): 11–15.

Hoga CA, Almeida FL, Reyes FGR (2018) A review on the use of hormones in fish farming: Analytical methods to determine their residues. Journal of Applied Aquaculture 30(4): 288–309. https://doi.org/10.1080/19476337.2018.1475423

Huervana FH, Dionela CS, de la Torre ED, del Castillo CS, Traifalgar RF (2022) Utilization of marine diatom *Thalassiosira weissflogii* as a feed additive in seawater-tolerant Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758) strain. Frontiers in Sustainable Food Systems 6: e1052951. https://doi.org/10.3389/fsufs.2022.1052951

Intoy MM, Traifalgar RF (2021) Feed value of fermented copra meal as a sustainable feed ingredient in the diet of saline-tolerant Nile tilapia *Oreochromis niloticus* (Linnaeus 1758). Journal of Sustainability Science and Management 16(8): 28–43. https://doi.org/10.46754/jssm.2021.12.003

Islam ZM, Sarder RI, Akhand RI (2015) Growth performance of genetically male tilapia derived from YY male, sex reversed male tilapia and mixed sex tilapia of *Oreochromis niloticus* in earthen pond aquaculture system in Bangladesh. International Journal of Fisheries and Aquatic Studies 2(3): 186–191.

Jiang DN, Kuang ZY, Yang KS, Huang YQ, Mustapha UF, Guo XZ, et al. (2022) Polymorphism in a sex-linked DNA marker located on LG23 in Hainan strain of Nile tilapia (*Oreochromis niloticus*). Journal of the World Aquaculture Society 53: 205–223. https://doi.org/10.1111/jwas.12815

Kohinoor AHM, Islam MS, Mazid MA, Hussain MG (2003) Breeding biology and monosex male seed production of Genetically Improved Farmed Tilapia (GIFT) strain of *Oreochromis niloticus* L. in Bangladesh. Bangladesh Journal of Fisheries Research 7(2): 105–114.

Lee BY, Penman DJ, Kocher TD (2003) Identification of a sex-determining region in Nile tilapia (*Oreochromis niloticus*) using bulked segregant analysis. Animal Genetics 34(5): 379–383. https://doi.org/10.1046/j.1365-2052.2003.01035.x

Leng STK, Weng CN, Tan ML, Wee ST, Samat N (2020) Consumer awareness and acceptance of aquaculture practices and products in Penang, Malaysia. In: Samat N (Ed.) Innovation and transformation in humanities for a sustainable tomorrow, vol. 89. European Proceedings of Social and Behavioural Sciences, 730–741. https://doi.org/10.15405/epsbs.2020.10.02.68

Liu F, Sun F, Yue GH (2025) Generating YY supermales and validating amh as the sex-determining gene in salt-tolerant pink tilapia. Aquaculture (Amsterdam, Netherlands) 613(1): 743305. https://doi.org/10.1016/j.aquaculture.2025.743305

Mair GC, Abucay JS, Skibinski DOF, Abella TA, Beardmore JA (1997) Genetic manipulation of sex ratio for the large-scale production of all-male tilapia, *Oreochromis niloticus*. Canadian Journal of Fisheries and Aquatic Sciences 54(2): 396–404. https://doi.org/10.1139/f96-282

Opiyo MA, Obiero KO, Abwao J, Awuor FJ, Kyule DN, Munguti JM (2020) Comparative growth performance of genetically male,

sex-reversed, and mixed-sex Nile tilapia (*Oreochromis niloticus*) reared in earthen ponds in Sagana, Kenya. Aquaculture Studies 21(1): 23–30. https://doi.org/10.4194/2618-6381-v21_1_03

Palaiokostas C, Bekaert M, Khan MGQ, Taggart JB, Gharbi K, McAndrew BJ, et al. (2013) Correction: Mapping and validation of the major sex-determining region in Nile tilapia (*Oreochromis niloticus* L.) using RAD sequencing. PLoS One 8(9): e76556. https://doi.org/10.1371/annotation/6320d31e-a818-445a-9c44-77a2ba5fe8e0

PCAARRD [Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development] (2024) Tilapia aquaculture to extend both in brackishwater and estuarine environments. PCAARRD, Los Baños. https://www.pcaarrd.dost.gov.ph

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H (2020) The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biology 18(7): e3000410. https://doi.org/10.1371/journal.pbio.3000410

Piferrer F (2001) Endocrine sex control strategies for the feminization of teleost fish. Aquaculture (Amsterdam, Netherlands) 197(1–4): 229–281. https://doi.org/10.1016/S0044-8486(01)00589-0

Rahman MM, Sarder MRI (2010) Production of hormone-induced supermale of Genetically Improved Farmed Tilapia (GIFT-YY) in Bangladesh. Asian Fisheries Science 23(2): 136–144. https://doi.org/10.33997/j.afs.2010.23.2.002

Shamsuddin M, Hossain MB, Rahman MM, Asadujjaman M, Ali MY (2012) Performance of monosex fry production of two Nile tilapia strains: GIFT and NEW GIPU. World Journal of Fish and Marine Sciences 4(1): 68–72.

Specker JL, Chandlee MK (2003) Methodology for estradiol treatment in marine larval and juvenile fish: Uptake and clearance in summer flounder. Aquaculture (Amsterdam, Netherlands) 217: 663–672. https://doi.org/10.1016/S0044-8486(02)00424-6

Sun YL, Jiang DN, Zeng S, Chu HJ, Ye K, Yang C, Yang SJ, Li MH, Wang DS (2014) Screening and characterization of sex-linked DNA markers and marker-assisted selection in the Nile tilapia (*Oreochromis niloticus*). Aquaculture (Amsterdam, Netherlands) 433: 19–27. https://doi.org/10.1016/j.aquaculture.2014.05.035

Triay C, Sissao R, Bezault E, D'Cotta H, Baroiller JF, Chevassus B, Conte MA (2022) Polymorphism of sex determination amongst wild populations suggests its rapid turnover within the Nile tilapia species. Frontiers in Genetics 13: e820772. https://doi.org/10.3389/fgene.2022.820772