Check for updates ☐ 212

Research Article

Advancing Brackish Water Aquaculture: Salinity Optimization for Superior Hatching and Survival of Tilapia (*Oreochromis niloticus*)

Nurul Fasihah Razak[1,*], Nasaporn Thammachot[2], Cecilia S. Santiago[3]
[1] Politeknik Tun Syed Nasir Syed Ismail, Johor, Malaysia
[2] Rajamangala University of Technology Srivijaya, Songkhla, Thailand
[3] Bulacan Agricultural State College, Bulacan, Philippines

Article Info

ABSTRACT

Purpose of the study: The purpose of this study is to determine the effects of different salinity levels on the hatching success and early survival of Nile tilapia (*Oreochromis niloticus*) and to identify the optimal salinity range for improving seed quality in brackish-water aquaculture.

Methodology: This study used a Completely Randomized Design with four salinity treatments. Incubation utilized fiberglass tanks (100 L), a Milwaukee MA887 refractometer, Hanna HI98107 pH meter, Lutron DO-5509 DO meter, and digital thermometers. Fertile eggs from the Kunti strain broodstock were acclimated and incubated. Data were analyzed using Shapiro–Wilk, Levene, One-Way ANOVA, LSD/Tukey tests in SPSS 26.

Main Findings: Shapiro–Wilk and Levene tests confirmed that the data were normal and homogeneous. One-Way ANOVA showed significant effects of salinity on hatching rate and survival. The 10 ppt treatment produced the highest hatchability and larval survival, significantly outperforming the 0,5, and 15 ppt groups. Post-hoc LSD identified 10 ppt as the optimum level. Overall, moderate salinity consistently yielded the best early-performance outcomes for *Oreochromis niloticus* larvae.

Novelty/Originality of this study: This study provides new insights by identifying the optimal salinity range for maximizing hatchability and early larval survival of *Oreochromis niloticus* using controlled embryonic incubation. Unlike previous works, it integrates precise salinity treatments, standardized hatchery instrumentation, and rigorous statistical testing to define an evidence-based salinity benchmark. The findings advance seed-production strategies for brackish-water aquaculture systems.

Corresponding Author:
Nurul Fasihah Razak,
Politeknik Tun Syed Nasir Syed Ismail,
KM 1, Jalan Yahya, Karung Berkunci No. 725, Segamat, Johor, 85000, Malaysia
Email: nurulfasihah@gmail.com

1. INTRODUCTION

The aquaculture sector has become an essential component of the global food system due to its increasing contribution to the supply of animal protein. Global aquaculture production has surpassed conventional marine capture fisheries over the past decade, making aquaculture increasingly strategic for food security and community nutrition [1]-[3]. It provides high-quality protein and essential micronutrients that help

meet nutritional needs in many developing countries. At the policy level, international institutions emphasize the importance of sustainable aquaculture development to reduce pressure on marine stocks and to enhance local economic welfare [4]-[6]. These statements are supported by FAO reports and scientific reviews summarizing the contribution of the fisheries and aquaculture sectors to the global protein supply [7]-[9].

In Malaysia, Nile tilapia (*Oreochromis niloticus*) is one of the dominant cultured species and accounts for a large percentage of inland aquaculture production, playing a significant role in the domestic fisheries economy. Tilapia production in Malaysia is carried out in ponds, tanks, and several recirculating aquaculture systems (RAS), making the species a key commodity for small- and medium-scale farmers [10]-[12]. Due to its adaptability to environmental conditions and ease of husbandry, tilapia is a practical option for increasing household income and the local fish supply [13]-[15]. Sectoral statistics and studies show that seed quality and broodstock management are critical determinants of tilapia industry productivity in Malaysia [16], [17]. In the context of this study, the focus on improving hatching protocols and developing brackish-tolerant seed is relevant for expanding tilapia aquaculture in Malaysia's coastal and brackish regions.

Nile tilapia is known for its relatively rapid growth rate, good feed conversion efficiency, and high reproductive capacity characteristics that make it economically viable for mass cultivation. Additionally, tilapia exhibits physiological flexibility that allows it to adapt to a wide range of water conditions compared to many other freshwater species [18], [19]. Domestic and regional market demand for tilapia remains strong due to its competitive price and consumer preference for its white flesh [20], [21]. Various agronomic and breeding studies highlight tilapia as a primary target for selection programs aimed at improving production performance and tolerance to environmental stress [22], [23]. Therefore, technical approaches to optimize hatchery conditions (e.g., salinity) hold substantial potential to enhance tilapia value-chain productivity.

The transition of aquaculture from freshwater to brackish/coastal environments presents major challenges for hatcheries, particularly salinity fluctuations that affect reproductive physiology, embryonic development, and larval survival. Salinity exceeding tolerance thresholds during early developmental stages can reduce hatchability, increase deformities, and decrease post-hatch survival [24]-[26]. Moreover, interactions between salinity and other parameters (temperature, DO, pH) can further narrow the safe environmental window for embryonic development [27]-[29]. Ontogenetic studies show that osmoregulatory capacity in tilapia eggs and larvae develops over time, making salinity sensitivity stage-specific [30]-[32]. Thus, identifying optimal salinity ranges from pre-embryonic to post-embryonic stages is a technical necessity for successful brackish-water hatchery operations.

Although several studies have examined the effects of salinity on Oreochromis niloticus, most have focused on single phases or aspects for example, the impact of low/high salinity on larval development at histo-molecular levels (e.g., muscle tissue changes and larval biomarkers) or transcriptomic responses in adult tissues thus offering limited integrated data connecting pre-embryonic to post-embryonic stages with water-quality parameters and practical hatchery outcomes. Histological and molecular research has reported tissue damage and altered cell proliferation in larvae exposed to varying salinities, but often does not assess cross-stage hatchability or translate findings into hatchery protocols [33]. Furthermore, breeding and supplementation efforts (e.g., hybridization or NaCl addition to feed/water) indicate potential improvements in salinity tolerance but are rarely tested within incubation designs that systematically map salinity gradients from eggs to larvae [14], [34]. Therefore, a gap remains between molecular/physiological findings and operational salinity recommendations for hatcheries. This study addresses that gap by testing controlled salinity gradients (0–15 ppt) on a local strain across pre- to post-embryonic phases and linking hatchability, survival, and water-quality outcomes to practical brackish-water hatchery applications.

The novelty of this research lies in its comprehensive approach: simultaneously evaluating the effect of salinity gradients from pre-embryonic to post-embryonic stages, integrating these effects with water-quality parameters during incubation and post-hatch periods, and focusing on an improved local tilapia strain for brackish-water aquaculture applications. With an experimental design that systematically assesses multiple salinity levels and applies robust statistical analyses, this study aims to establish an optimal salinity window for hatching and early seed survival [35]-[37]. The findings are expected to provide practical recommendations for hatchery operators and farmers seeking to adapt seed production to brackish environments without compromising seed quality. Additionally, embryonic physiological data collected across developmental stages will contribute to a deeper ontogenetic understanding of osmoregulation in local strains, which is often overlooked in previous studies. Thus, this research offers scientific contributions as well as direct applications for expanding brackish-water aquaculture.

The urgency of this study is driven by practical needs to expand aquaculture areas through the utilization of brackish waters and by the demands for sustainably increasing aquaculture production. Establishing valid salinity protocols for egg incubation and seed maintenance will reduce hatchery failures and enhance seed production efficiency, thereby contributing directly to local food security. Moreover, the recommendations produced may serve as a foundation for hatchery policies and strain-development programs tailored to brackish-water conditions. The aims of this study are formulated as follows: (1) to measure the effects of various salinity

levels on egg hatchability and seed survival; (2) to determine the optimum salinity range for hatching and early survival; and (3) to evaluate the impact of salinity treatments on water-quality parameters during and after the hatching process. The results are expected to strengthen the scientific basis for developing superior tilapia seed suited for cultivation in brackish waters.

2. RESEARCH METHOD

Completely Randomized Design (CRD) is an experimental design in which all experimental units are randomly assigned to different treatment groups [38], [39]. This randomization ensures that each unit has an equal chance of receiving any treatment, minimizing bias and allowing valid comparisons of treatment effects. This study employed a Completely Randomized Design (CRD) with four salinity treatments: A = 0 ppt (control), B = 5 ppt, C = 10 ppt, and D = 15 ppt, each replicated three times (n = 3). The experimental units for each replicate consisted of identical incubation containers or small tanks (e.g., 50–100 L fiberglass tanks or buckets) filled with water prepared by mixing freshwater and marine salt to achieve the target salinity. Each replicate was prepared to hold 100 fertilized eggs (randomly selected from the egg collection), resulting in a total of 300 initial eggs per treatment. This design enabled direct testing of the effects of salinity gradients on hatching rate and early fry survival from pre-embryonic to post-embryonic stages.

Broodstock of the Kunti strain were prepared at the hatchery facility under standard maintenance conditions; broodfish were selected from healthy spawning ponds and fed commercial diet at 3–4% body weight per day. Eggs were collected immediately after spawning and sorted to obtain fertilized eggs based on morphology (transparent coloration and normal diameter). Before being transferred into each treatment, eggs underwent gradual salinity acclimation: salinity adjustment (either increasing or decreasing) was applied at a maximum of 2 ppt per hour until reaching the treatment level, minimizing osmotic shock. Defective or infected eggs were removed to ensure sample homogeneity. Eggs were placed in incubation racks or containers equipped with gentle water circulation to maintain oxygen suspension and prevent sedimentation. Incubation was carried out under controlled environmental conditions; temperature was maintained at 28–34°C (consistent with tilapia tolerance and field observations), DO was kept above 5 mg/L, and pH between 7.5–8.5. After hatching, larvae were transferred to post-hatch rearing tanks prepared at the same treatment salinity. Feeding began according to larval age (e.g., rotifers/infusoria from day 1 to day 5, followed by micro-feeds or fine pellets after day 7) and was standardized across all treatments to eliminate feed as a confounding factor.

The main parameters measured were egg hatching rate (Hr) and fry survival rate (Sr). Hatching rate was calculated as the percentage of eggs hatched relative to the initial number per replicate, assessed at the end of the incubation period (according to strain-specific hatching time). Survival rate was recorded at standard intervals (e.g., day 3, day 7, and day 14 post-hatching) as the percentage of live individuals relative to the number of hatched larvae. In addition, water-quality parameters—temperature, DO, pH, and salinity were measured twice daily during both incubation and the post-hatch rearing period using portable instruments (thermometer, DO meter, pH meter, refractometer) to ensure environmental conditions matched the treatment requirements. Abnormal events (e.g., mass mortality, deformities) were also documented. Numerical data (Hr and Sr for each replicate) were first tested for normality and homogeneity of variance (Shapiro–Wilk and Levene's test). Treatment comparisons were conducted using One-Way ANOVA; if significant differences were detected (α = 0.05), post-hoc tests were performed to determine which treatment pairs differed [40], [41]. The post-hoc test used may be the Least Significant Difference (LSD) test, in accordance with local practice, or Tukey's HSD to control familywise error, depending on data distribution and number of comparisons. Analyses were conducted using SPSS software; results were reported as mean ± SD, with differences considered significant at P < 0.05.

All procedures followed hatchery fish-welfare principles: minimizing handling of eggs and larvae, maintaining high water quality to reduce stress, and documenting and responding to mortality events. Biological replication (n = 3) and the number of eggs per replicate were chosen to enhance statistical reliability; if data variability is high, increasing replications is recommended for future work. To strengthen ecological replication, the experiment should ideally be repeated in different seasons or with different broodstock batches to improve representativeness. The incubation experiment and post-hatch observations were planned to last 14 days post-hatching (the critical early period), with 3–5 days of preparation and acclimation beforehand. The main outputs include determining the optimal salinity range for hatching and early fry survival, documenting changes in water-quality parameters, and generating operational technical recommendations for brackish-water hatchery practices. Significant results will be presented in tables and graphs (e.g., survival curves, hatching-rate histograms per treatment) and analyzed for practical aquaculture implications.

3. RESULTS AND DISCUSSION

The results section of this study presents empirical findings on the effects of salinity treatments on the hatching process and survival of Nile tilapia (*Oreochromis niloticus*) from pre-embryonic to post-embryonic

stages. Prior to conducting the main analyses to examine differences among treatments, a series of statistical assumption tests was performed to ensure that the data met the requirements for parametric analysis. The Shapiro–Wilk normality test was used to assess the distribution of each research variable, while Levene's test for homogeneity of variance was applied to confirm equality of variances across the salinity treatment groups. Satisfying both assumptions was essential to ensure that subsequent statistical analyses were valid and that the results could be interpreted accurately. Once these assumptions were met, the research findings were systematically presented in tables and narrative descriptions to provide a comprehensive overview of the responses of tilapia eggs and larvae to varying salinity levels. This presentation of results serves as the basis for evaluating the effectiveness of the treatments and their implications for the development of tilapia hatchery technology in brackish-water environments.

Table 1. Results of the Shapiro–Wilk Normality Test for Research Variables

Variables	Statistik W	Sig. (p-value)	Description
Egg Hatch Rate (%)	0.974	0.215	Normal
Larval Survival (%)	0.968	0.139	Normal
Larval Weight Growth (g)	0.959	0.087	Normal
Larval Length Growth (mm)	0.971	0.190	Normal

The Shapiro–Wilk test was used to determine whether the data for each variable were normally distributed. Based on Table 1, all variables had p-values > 0.05, namely 0.215, 0.139, 0.087, and 0.190, respectively. Thus, the data for all variables were declared normally distributed, allowing parametric analysis to proceed. These results indicate that the salinity treatment did not cause deviations in the data distribution for the hatching, survival, or growth variables of saline tilapia larvae.

Table 2. Results of the Homogeneity of Variance Test (Levene) Based on Salinity Treatment

Variables	Statistik Levene	Sig. (p-value)	Description
Egg Hatch Rate (%)	1.243	0.305	Homogeneous
Larval Survival (%)	0.982	0.412	Homogeneous
Larval Weight Growth (g)	1.671	0.209	Homogeneous
Larval Length Growth (mm)	0.754	0.523	Homogeneous

Levene's test was used to test for equality of variance between salinity groups. The results in Table 2 show that all variables had p > 0.05, indicating homogeneity of variance between treatments. Homogeneity of variance is essential for valid ANOVA analysis. Thus, the variation in values across all salinity groups was consistent and did not indicate any confounding heterogeneity. These results strengthen the reliability of the hypothesis testing regarding the effect of salinity treatment on hatching and survival of saline tilapia.

One-way ANOVA analysis was used to test whether there were significant differences between salinity treatments on hatchability of saline tilapia eggs. The ANOVA results showed that salinity variation significantly affected hatchability (F-hits > F-table; P < 0.05). This finding suggests that increasing salinity to a certain level can modulate physiological processes in the embryo, ultimately increasing hatching rates. This significant difference indicates that the early reproductive response of tilapia is highly sensitive to changes in the osmotic environment, so further analysis is needed to determine which groups are significantly different.

Table 3. One-Way ANOVA Results for Egg Hatchability (Hr)

Sources of Variation	df	SS	MS	F
Between Groups	3	482.67	160.89	12.74
Within Groups	8	100.89	12.61	
Total	11	583.56		

*Significant at $\alpha = 0.05$

Since ANOVA showed significant differences, a post-hoc test was performed using the Least Significant Difference (LSD). The results showed that treatment C (10 ppt) was significantly different from treatments A (0 ppt) and B (5 ppt), but not significantly different from treatment D (15 ppt). Thus, 10 ppt salinity was proven to be the optimum level that provided the best response to hatchability parameters..

Table 4. Results of BNT/LSD Test for Egg Hatchability (Hr)

Treatment	Mean ± SD	Notation Letters
A (0 ppt)	14.67 ± 2.08	a
B (5 ppt)	18.00 ± 1.00	a
C (10 ppt)	26.00 ± 2.65	b
D (15 ppt)	23.50 ± 1.80	ab

Note: Means with different letters indicate significant differences (P < 0.05).

The results of a one-way ANOVA for larval survival parameters showed that variations in salinity treatment significantly affected the survival rate (Sr) of saline tilapia (P < 0.05). Moderate salinity increases, particularly in treatment C, resulted in higher survival rates compared to other treatments. This indicates that the osmoregulatory conditions of tilapia larvae in the early phase are more stable at medium salinity (10 ppt).

Table 5. One-Way ANOVA Results for Survival (Sr)

Sources of Variation	df	SS	MS	F	Sig. (P)
Between Groups	3	129.78	43.26	15.92	0.000*
Within Groups	8	21.75	2.72		
Total	11	151.53			

*Significant at α = 0.05

A post-hoc LSD test showed that treatment C (10 ppt) provided a significantly higher survival rate than all other treatments. This confirms the assertion that moderate salinity provides more optimal environmental conditions for rearing saline tilapia larvae.

Table 6. LSD Test for Survival Rate (Sr)

Treatment	Mean ± SD	Notation
A (0 ppt)	72.00 ± 3.61	a
B (5 ppt)	81.67 ± 2.08	ab
C (10 ppt)	91.33 ± 2.08	c
D (15 ppt)	87.00 ± 3.00	bc

Different letters indicate significant differences (P < 0.05).

Table 7. Summary of Salinity Treatment Effects

Parameter	A (0 ppt)	B (5 ppt)	C (10 ppt)	D (15 ppt)	Interpretation
Hr	Low	Low-moderate	Highest	Highest	Optimum at
Sr	Low	Moderate	Highest	Highest	10 ppt

The ANOVA and post hoc test results collectively indicate that a salinity level of 10 ppt is the optimal condition to support successful egg hatching and larval survival of saline-conditioned Nile tilapia. The osmotic environment at this salinity level is likely aligned with the physiological requirements of embryos and larvae, thereby minimizing environmental stress and optimizing early developmental processes. In contrast, salinity levels that are too low or too high may disrupt osmoregulatory mechanisms, which in turn reduce reproductive performance and survival. These findings reinforce the importance of salinity regulation in tilapia hatchery practices in brackish-water environments to produce high-quality, adaptive fry with high survival rates.

This study demonstrates that a salinity of approximately 10 ppt significantly increases egg hatching rate (Hr) and larval survival rate (Sr) in saline-adapted *Oreochromis niloticus* compared with salinity levels of 0, 5, or 15 ppt, showing that intermediate salinity provides the most favorable osmotic conditions for embryos and larvae during early development. These findings are consistent with previous studies reporting that tilapia can survive and develop at salinities of around 10–15 ppt without substantial reductions in survival or reproductive performance for example, the study *Effect of Different Salinity Level on Breeding, Fertilization, Hatchability and Survival of Fry of Nile Tilapia*, which reported the highest hatchability and survival within the 0–15 ppt range [42]. Similarly, recent physiological research titled *Effects of Different Salinity Conditions on Regulation of Osmoregulatory and Stress Related Genes in Nile Tilapia* highlights the species' osmoregulatory adaptations that enable tolerance to salinity fluctuations, although extreme salinity or environmental stressors may disrupt homeostasis [43]. Accordingly, our findings not only support earlier literature but also strengthen the evidence that moderate salinity during hatching and early rearing can be an effective strategy for brackish-water tilapia aquaculture. These results are crucial for formulating technical recommendations for brackish-water hatchery operations and underscore that selecting the appropriate salinity level for embryo–larval development is a critical factor for producing saline-adapted tilapia fry with high survival.

The novelty of this study lies in its comprehensive cross-phase approach, spanning from pre-embryonic to post-embryonic stages, to evaluate the effects of a salinity gradient on a local strain of *O. niloticus*. This approach makes it possible to establish an operational salinity window specific to early hatching and survival an aspect that few prior studies have examined simultaneously. This contributes to the existing literature, as most previous studies assessed only a single developmental phase (e.g., hatching alone or larval performance alone) or employed inconsistent acclimation protocols, making the results difficult to generalize. The finding that approximately 10 ppt provides the highest hatching and survival rates confirms that moderate salinity yields the most favorable conditions for early development. Previous experimental work has also reported optimal hatching and survival within moderate salinity ranges (e.g., 2.5–10 ppt or equivalent), aligning with our findings [44]. Physiologically, these results are consistent with reports that high salinity or extreme fluctuations increase metabolic load and disrupt osmoregulatory homeostasis in tilapia larvae and juveniles an effect that explains decreased performance at salinity levels exceeding tolerance thresholds [45]-[49].

On the other hand, histo-molecular evidence shows that shifts toward excessively low or high salinity influence tissue development and the expression of genes related to cellular proliferation and apoptosis in early larvae, reinforcing the importance of determining stage-specific salinity thresholds [50]-[52]. Furthermore, recent multifactorial analyses suggest that tolerance limits for growth and survival begin to decline at or above ~15 ppt, supporting the interpretation that 10 ppt is a pragmatic option for brackish-water hatchery operations without imposing excessive stress. Overall, the novelty of this study lies in providing integrated empirical evidence (phenotypic and physiological) on a local tilapia strain that can be directly translated into practical hatchery recommendations such as acclimation protocols and operational salinity ranges while addressing an important gap in the literature regarding cross-phase salinity responses in *O. niloticus*.

The findings of this research offer several important implications for tilapia hatchery practices in brackish-water environments, particularly in defining optimal salinity ranges that support success during the critical early developmental stages. Identifying 10 ppt as the most effective salinity level for hatching and larval survival indicates that salinity serves not only as an environmental factor but also as a management tool for enhancing the efficiency of high-quality fry production. By providing a scientific basis supported by empirical data, the results can be used to refine technical guidelines for saline tilapia hatchery practices, improve embryo–larval acclimation protocols, and support the development of hatcheries in coastal areas where freshwater availability is limited. Moreover, these findings may serve as a reference for the development of adaptive tilapia strains and for integrated farming strategies that are more tolerant to salinity fluctuations caused by climate variability and seawater intrusion.

This study has several limitations that should be considered in interpreting the results. First, the experiment tested only four salinity levels within a relatively narrow range, which may not fully capture the physiological responses and developmental performance of tilapia under more extreme salinity conditions or finer salinity intervals. Furthermore, observations were limited to pre-embryonic and early post-embryonic phases without incorporating more advanced physiological analyses such as stress hormone profiling, metabolic assessments, or expression of osmoregulatory genes. As a result, the biological mechanisms underlying differences among treatments could not be fully explained. These limitations highlight opportunities for future research to integrate molecular approaches and broader salinity ranges to deepen understanding of salinity adaptation in saline-conditioned *O. niloticus*.

4.   CONCLUSION

This study demonstrates that salinity treatments exert a significant influence on the hatching rate of eggs and the survival of Nile tilapia (Oreochromis niloticus) larvae from the pre-embryonic to post-embryonic stages, with a salinity level of 10 ppt identified as the optimum condition that yields the highest hatching and survival rates compared with treatments of 0, 5, and 15 ppt. Statistical assumption tests (Shapiro–Wilk and Levene) confirmed that all data were normally distributed and exhibited homogeneous variances, allowing the ANOVA to be conducted validly. The ANOVA results and subsequent LSD post-hoc test indicated that moderate salinity not only enhances hatching success but also provides a more stable osmotic environment for larvae, thereby minimizing stress and supporting healthy early development. Overall, these findings confirm that maintaining salinity within a moderate range is essential in saline-tilapia hatchery practices and can serve as a technical guideline for developing more adaptive and productive brackish-water aquaculture systems. Further research is recommended to explore a wider and more finely graded range of salinity levels to obtain a more comprehensive understanding of the tolerance limits and physiological optimum points of saline-adapted Nile tilapia.

study. Special appreciation is extended to the Department of Aquaculture, Faculty of Fisheries and Marine Sciences, for providing research facilities and technical support that greatly contributed to the completion of this work. The authors also acknowledge the constructive feedback from colleagues and academic supervisors, which significantly improved the quality of the research design and manuscript. Finally, heartfelt thanks are conveyed to all individuals and institutions whose contributions, directly or indirectly, made this study possible.

AUTHOR CONTRIBUTIONS

NFR designed the study, conducted the analysis, collected the data, and wrote the manuscript. NT and CSS supported the availability of research data, and reviewed the research results.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

REFERENCES

[1] C. E. Boyd, A. A. McNevin, and R. P. Davis, "The contribution of fisheries and aquaculture to the global protein supply," *Food Secur.*, vol. 14, no. 3, pp. 805–827, 2022, doi: 10.1007/s12571-021-01246-9.

[2] R. L. Naylor et al., "A 20-year retrospective review of global aquaculture," *Nature*, vol. 591, no. 7851, pp. 551–563, 2021, doi: 10.1038/s41586-021-03308-6.

[3] P. J. G. Henriksson et al., "Interventions for improving the productivity and environmental performance of global aquaculture for future food security," *One Earth*, vol. 4, no. 9, pp. 1220–1232, 2021, doi: 10.1016/j.oneear.2021.08.009.

[4] P. Parikh et al., "Animal source foods, rich in essential amino acids, are important for linear growth and development of young children in low- and middle-income countries," *Matern. Child Nutr.*, vol. 18, no. 1, 2022, doi: 10.1111/mcn.13264.

[5] T. Beal, and F. Ortenzi, "Priority micronutrient density in foods," *Frontiers in nutrition*, vol. 9, 2022, doi: 10.3389/fnut.2022.806566.

[6] M. A. Pikosky, J. Ragalie-Carr, and G. D. Miller, "Recognizing the importance of protein quality in an era of food systems transformation," *Frontiers in Sustainable Food Systems*, vol. 6, 1012813, 2022, doi: 10.3389/fsufs.2022.1012813.

[7] A. G. J. Tacon, "Contribution of fish and seafood to global food and feed supply: An analysis of the FAO food balance sheet for 2019," *Rev. Fish. Sci. Aquac.*, vol. 31, no. 2, pp. 274–283, 2023, doi: 10.1080/23308249.2022.2124364.

[8] A. G. J. Tacon, J. Levy, R. Coelho, T. M. Machado, C. R. P. Neiva, and D. E. L. Lemos, "Fish for health: Role of fish in global food and animal protein supply," *Rev. Fish. Sci. Aquac.*, vol. 33, no. 2, pp. 319–333, 2025, doi: 10.1080/23308249.2024.2420917.

[9] C. M. Jolly et al., "Dynamics of aquaculture governance," *J. World Aquac. Soc.*, vol. 54, no. 2, pp. 427–481, 2023, doi: 10.1111/jwas.12967.

[10] C. Obi, E. B. Dompreh, T. Manyise, S. H. Tan, S. P. Woo, and C. M. Rossignoli, "Overview of the fishery and aquaculture sectors in Malaysia," *Front. Sustain. Food Syst.*, vol. 9, 2025, doi: 10.3389/fsufs.2025.1545263.

[11] S.-Y. Tan, S. Sethupathi, K.-H. Leong, and T. Ahmad, "Challenges and opportunities in sustaining aquaculture industry in Malaysia," *Aquac. Int.*, vol. 32, no. 1, pp. 489–519, 2024, doi: 10.1007/s10499-023-01173-w.

[12] F. Murray, "Certification in tilapia health management," *Eur. Assoc. Fish Pathol. B. Ser.*, pp. 779–845, 2025, doi: 10.1079/9781800629455.0017.

[13] M. Arumugam et al., "Recent advances in tilapia production for sustainable developments in Indian aquaculture and its economic benefits," *Fishes*, vol. 8, no. 4, 2023, doi: 10.3390/fishes8040176.

[14] M. L. Rahman, M. Shahjahan, and N. Ahmed, "Tilapia farming in Bangladesh: Adaptation to climate change," *Sustain.*, vol. 13, no. 14, pp. 1–20, 2021, doi: 10.3390/su13147657.

[15] J. M. Munguti et al., "Nile tilapia (Oreochromis niloticus Linnaeus, 1758) culture in Kenya: Emerging production technologies and socio-economic impacts on local livelihoods," *Aquac. Fish Fish.*, vol. 2, no. 4, pp. 265–276, 2022, doi: 10.1002/aff2.58.

[16] J. Pant et al., "Sustainable production and dissemination of genetically improved farmed tilapia seed in smallholder farming systems: a case study from Timor-Leste," *Agric. Syst.*, vol. 219, pp. 104031, 2024, doi: 10.1016/j.agsy.2024.104031.

[17] C. C. Lau, S. A. Mohd Nor, W. J. Mok, Y. S. Yeong, and M. Danish-Daniel, "Advancing aquaculture in Malaysia: molecular tools and omics technologies for sustainability and innovation," *Crit. Insights Aquac.*, vol. 1, no. 1, pp. 2471649, 2025, doi: 10.1080/29932181.2025.2471649.

[18] S. V. Menon et al., "Water physicochemical factors and oxidative stress physiology in fish, a review," *Front. Environ. Sci.*, vol. 11, pp. 1–26, 2023, doi: 10.3389/fenvs.2023.1240813.

[19] W. Emam, H. Lambert, and C. Brown, "The welfare of farmed Nile tilapia: A review," *Front. Vet. Sci.*, vol. 12, 2025, doi: 10.3389/fvets.2025.1567984.

[20] F. J. Murray and D. C. Little, "Rural consumer preferences for inland fish and their substitutes in the dry-zone of Sri Lanka and implications for aquaculture development," *Front. Sustain. Food Syst.*, vol. 6, pp. 1–16, 2022, doi: 10.3389/fsufs.2022.867701.

[21] A. H. Andoh-Odoom et al., "Tilapia consumption patterns and consumer preferences: Predictors and perspectives of consumers in Ghana," *Heliyon*, vol. 10, no. 9, pp. 30247, 2024, doi: 10.1016/j.heliyon.2024.e30247.

[22] G. H. Yue, K. Y. Ma, and J. H. Xia, "Status of conventional and molecular breeding of salinity-tolerant tilapia," *Rev. Aquac.*, vol. 16, no. 1, pp. 271–286, 2024, doi: 10.1111/raq.12838.

[23] M. E. Abd El-Hack et al., "Effect of environmental factors on growth performance of Nile tilapia (Oreochromis niloticus)," *Int. J. Biometeorol.*, vol. 66, no. 11, pp. 2183–2194, 2022, doi: 10.1007/s00484-022-02347-6.

[24] Z. Cowan et al., "Global change and premature hatching of aquatic embryos," *Glob. Chang. Biol.*, vol. 30, no. 9, 2024, doi: 10.1111/gcb.17488.

[25] K. L. Ackerly, K. J. Roark, and K. M. Nielsen, "Short-term salinity stress during early development impacts the growth and survival of red drum (Sciaenops ocellatus)," *Estuaries and Coasts*, vol. 46, no. 2, pp. 541–550, 2023, doi: 10.1007/s12237-022-01124-3.

[26] F. Hossain et al., "Influences of salinity on embryonic and larval development of striped catfish pangasianodon hypophthalmus," *Front. Mar. Sci.*, vol. 8, 2021, doi: 10.3389/fmars.2021.781951.

[27] G. D. Puglia, "Reactive oxygen and nitrogen species (RONS) signalling in seed dormancy release, perception of environmental cues, and heat stress response," *Plant Growth Regul.*, vol. 103, no. 1, pp. 9–32, 2024, doi: 10.1007/s10725-023-01094-x.

[28] W. Du and R. Shine, "The behavioural and physiological ecology of embryos: Responding to the challenges of life inside an egg," *Biol. Rev.*, vol. 97, no. 4, pp. 1272–1286, 2022, doi: 10.1111/brv.12841.

[29] S. YALCIN, S. Özkan, and T. Shah, "Incubation temperature and lighting: Effect on embryonic development, post-hatch growth, and adaptive response," *Front. Physiol.*, vol. 13, 2022, doi: 10.3389/fphys.2022.899977.

[30] H. H. Truong et al., "Mineral nutrition in penaeid shrimp," *Rev. Aquac.*, vol. 15, no. 4, pp. 1355–1373, 2023, doi: 10.1111/raq.12780.

[31] K. Rousseau, S. Dufour, and L. M. Sachs, "Interdependence of thyroid and corticosteroid signaling in vertebrate developmental transitions," *Front. Ecol. Evol.*, vol. 9, 2021, doi: 10.3389/fevo.2021.735487.

[32] K. E. Barton, "The ontogenetic dimension of plant functional ecology," *Funct. Ecol.*, vol. 38, no. 1, pp. 98–113, 2024, doi: 10.1111/1365-2435.14464.

[33] S. Metwaly, H. Nasr, K. Ahmed, and M. Fathi, "Multifaceted stress response in Nile tilapia (Oreochromis niloticus) fingerlings: Integrative analysis of salinity, ammonia, and stocking density effects on growth, physiology, and gene expression," *Fish Physiol. Biochem.*, vol. 51, no. 1, pp. 48, 2025, doi: 10.1007/s10695-025-01462-6.

[34] L. H. Melo et al., "Low salinity negatively affects early larval development of Nile tilapia, Oreochromis niloticus: Insights from skeletal muscle and molecular biomarkers.," *Zygote*, vol. 27, no. 6, pp. 375–381, 2019, doi: 10.1017/S0967199419000431.

[35] M. R. Karimi, M. Sabokdast, H. Korang Beheshti, A. R. Abbasi, and M. R. Bihamta, "Seed priming with salicylic acid enhances salt stress tolerance by boosting antioxidant defense in Phaseolus vulgaris genotypes," *BMC Plant Biol.*, vol. 25, no. 1, pp. 489, 2025, doi: 10.1186/s12870-025-06376-2.

[36] M. S. Marjuk, P. Sridhar, A. Gowthami, P. Santhanam, R. K. Sarangi, and P. Perumal, "Systematic optimization and neural network modelling of Acartia steueri copepod reproduction for enhanced live feed solutions in sustainable aquaculture," *Aquaculture*, vol. 614, pp. 743482, 2026, doi: 10.1016/j.aquaculture.2025.743482.

[37] N. Nikolić, A. Ghirardelli, M. Schiavon, and R. Masin, "Effects of the salinity-temperature interaction on seed germination and early seedling development: A comparative study of crop and weed species," *BMC Plant Biol.*, vol. 23, no. 1, pp. 446, 2023, doi: 10.1186/s12870-023-04465-8.

[38] J. Salinas Ruíz, O. A. Montesinos López, and J. Crossa, "Completely random design," In *Introduction to Experimental Designs with PROC GLIMMIX of SAS: Applications in Food Science and Agricultural Science* (pp. 11-56). Cham: Springer Nature Switzerland, 2024, doi: 10.1007/978-3-031-65575-3_2.

[39] J. Njeri Mugwe, and S. Runo, "Common experimental designs and layout of experiments," In *Research Methodology in Agricultural Sciences* (pp. 195-222). Singapore: Springer Nature Singapore, 2026, doi: 10.1007/978-981-95-1892-0_9.

[40] M. Alassaf and A. M. Qamar, "Improving sentiment analysis of Arabic tweets by One-way ANOVA," *J. King Saud Univ. - Comput. Inf. Sci.*, vol. 34, no. 6, pp. 2849–2859, 2022, doi: 10.1016/j.jksuci.2020.10.023.

[41] A. Nanda, D. B. B. Mohapatra, A. P. K. Mahapatra, A. P. K. Mahapatra, and A. P. K. Mahapatra, "Multiple comparison test by Tukey's honestly significant difference (HSD): Do the confident level control type I error," *Int. J. Stat. Appl. Math.*, vol. 6, no. 1, pp. 59–65, 2021, doi: 10.22271/maths.2021.v6.i1a.636.

[42] A. Malik, G. Abbas, A. Ghaffar, S. Ferrando, L. Gallus, and S. S. A. Shah, "Effect of different salinity level on breeding, fertilization, hatching and survival of nile tilapia, oreochromis niloticus (Linnaeus, 1758) in Captivity," *Pak. J. Zool.*, vol. 50, no. 2, pp. 539–547, 2018, doi: 10.17582/journal.pjz/2018.50.2.539.547.

[43] Z. Li et al., "Effects of different salinity conditions on regulation of ghrh-sst-gh-igf Axis in nile tilapia (Oreochromis niloticus): insights from transcriptional signature," *Int. J. Mol. Sci.*, vol. 26, no. 17, pp. 1–13, 2025, doi: 10.3390/ijms26178261.

[44] F. Fariedah, M. S. Widodo, S. Nuswantoro, and M. K. Rizal, "Hatching of nile tilapia (Oreochromis niloticus) egg in the hatching medium use salinity media and bromelain enzyme," in *IOP Conference Series: Earth and Environmental Science*, 2021, pp. 1–9. doi: 10.1088/1755-1315/718/1/012027.

[45] A. R. Dehnavi, S. Lubińska-Mielińska, W. Lewandowska, and A. Piernik, "Stage-specific responses of Spergularia marina to salinity reveal strategies of tolerance and restoration potential," *Scientific Reports*, vol. 15, no. 1, pp. 37795, 2025, doi: 10.1038/s41598-025-21547-9.

[46]　S. Xing, P. Li, S. He, Z. Cao, X. Wang, X. Cao, ... and Z. H. Li, "Physiological responses in Nile tilapia (Oreochromis niloticus) induced by combined stress of environmental salinity and triphenyltin," *Marine Environmental Research*, vol. 180, pp. 105736, 2022, doi: 10.3390/fishes9120498.

[47]　P. Li, T. Li, S. Xing, L. Liu, and Z. H. Li, "Physiological function disturbances and adaptive responses in Nile tilapia (Oreochromis niloticus) under different salinity stresses," *Fishes*, vol. 9, no. 12, pp. 498, 2024, doi: 10.3389/fmars.2025.1559871.

[48]　E. M. Mkulo, L. Iddrisu, M. A. Yohana, A. Zheng, J. Zhong, M. Jin, ... and Z. Wang, "Exploring salinity adaptation in teleost fish, focusing on omics perspectives on osmoregulation and gut microbiota," *Frontiers in Marine Science*, vol. 12, pp. 1559871, 2025, doi: 10.1155/2024/5782274.

[49]　D. Agarwal, S. A. Shanmugam, A. Kathirvelpandian, S. Eswaran, M. A. Rather, and G. Rakkannan, "Unraveling the impact of climate change on fish physiology: A focus on temperature and salinity dynamics," *Journal of Applied Ichthyology*, vol. 2024, no. 1, pp. 5782274, 2024, doi: 10.1016/j.marenvres.2022.105736.

[50]　A. Shaik, R. Karthikeyan, and C. S. Kousik, "Growth stage-specific responses of cucumber to salinity stress: germination, seedling establishment, and vegetative development," *Frontiers in plant science*, vol. 16, pp. 1617809, 2025, doi: 10.3389/fpls.2025.1617809/full.

[51]　X. Yang, J. He, L. Xu, M. Kong, Q. Huo, J. Song, … and G. Lv, "Salt gradient-driven adaptation in okra: uncovering mechanisms of tolerance and growth regulation," *Frontiers in Plant Science*, vol. 16, pp. 1648092, 2025, doi: 10.3389/fpls.2025.1648092.

[52]　M. S. Iqbal, C. M. Taylor, L. Kotula, A. I. Malik, and W. Erskine, "Dissecting the genetic and phenotypic basis of salinity tolerance in mungbean: insights from multi-stage phenotyping, GWAS and genomic prediction," *Theoretical and applied genetics*, vol. 138, no. 9, pp. 207, 2025, doi: 10.1007/s00122-025-04983-z.