

Effect of Stocking Density under 10‰ Salinity on Production Performance and Chemical Composition of Monosex Tilapia Fingerlings (*Oreochromis niloticus* × *O. aureus*)

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□ ABSTRACT □

This study aimed to evaluate the effect of stocking density on growth performance, survival rates, feed conversion efficiency and body chemical composition of the Monosex tilapia fingerlings (*Oreochromis niloticus* × *O. aureus*) cultured under low salinity (10‰).

The experiment lasted 73 days, during which fingerlings were randomly assigned to three treatments with three replicates: low density D1 (143 fish/m³), medium density D2 (286 fish/m³), and high density D3 (571 fish/m³). The fish were fed twice daily at a rate of 4–5% of their body weight.

The results showed significant differences ($P < 0.05$) among treatments in daily growth rate and feed conversion ratio. The highest numerical daily gain was recorded for D2 (0.199 g/day), while D1 and D2 did not differ significantly ($P > 0.05$); both were significantly higher than D3 (0.073 g/day). The best feed conversion ratio was observed in D1 (1.15), followed by D2 (1.31), with D3 recording the poorest value (3.10). Protein and lipid contents were lower in D3 compared to D1, while ash content was higher. Survival rates reached 100% in D1 and D2, but only 90% in D3. The study recommends that low density be adopted (143 fish/m³) as the optimal stocking density for culturing monosex tilapia fingerlings at a salinity of 10‰. To confirm these results, field trials are needed.

Keywords: Monosex tilapia, stocking density, low Salinity, Growth performance, Feed conversion ratio (FCR), Brackish-water aquaculture.

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تأثير كثافة الاستزراع تحت ملوحة 10% على الأداء الإنتاجي والتركيب الكيميائي لإصبعيات المشط وحيد الجنس (*Oreochromis niloticus* × *O. aureus*)

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□ ملخص □

هدفت هذه الدراسة إلى تقييم تأثير كثافة الاستزراع على كل من: معدل البقاء، أداء النمو، كفاءة التحويل الغذائي والتركيب الكيميائي للجسم الكامل لإصبعيات المشط وحيد الجنس Monosex Tilapia (*Oreochromis niloticus* × *O. aureus*) المستزرعة في ملوحة منخفضة 10%.

استمرت التجربة 73 يوماً، حيث وزعت الإصبعيات عشوائياً على ثلاث معاملات بثلاثة تكرارات: كثافة منخفضة D1 (143 سمكة/م³)، كثافة متوسطة D2 (286 سمكة/م³)، وكثافة عالية D3 (571 سمكة/م³). عُلِّقت الأسماك مرتين يومياً بمعدل 4-5% من وزن جسمها.

أظهرت النتائج فروقاً معنوية ($P < 0.05$) بين المعاملات في معدل النمو اليومي ومعامل التحويل الغذائي. سُجل أعلى معدل نمو يومي عددياً في المعاملة D2 (0.199 غ/يوم)، بينما لم تختلف D1 و D2 معنوياً ($P > 0.05$)؛ وتفاوتت كلتاهما معنوياً على D3 (0.073 غ/يوم). سُجل أفضل معامل تحويل غذائي في D1 (1.15)، يليه D2 (1.31)، بينما سجلت المعاملة D3 أسوأ قيمة (3.10). انخفض محتوى البروتين والدهون في D3 مقارنة بـ D1، بينما ارتفع محتوى الرماد. بلغ معدل البقاء 100% في D1 و D2، و 90% في D3. توصي الدراسة باعتماد الكثافة المنخفضة (143 سمكة/م³) ككثافة مثلى لاستزراع إصبعيات المشط وحيد الجنس تحت الملوحة 10%. ولتأكيد هذه النتائج، يلزم إجراء تجارب ميدانية.

الكلمات المفتاحية: المشط وحيد الجنس Monosex Tilapia، كثافة الاستزراع، ملوحة منخفضة، أداء النمو، معامل التحويل الغذائي (FCR)، الاستزراع بمياه مختلطة.



حقوق النشر : مجلة جامعة اللاذقية (تشرين سابقاً) - سورية، يحتفظ المؤلفون بحقوق النشر بموجب

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Introduction:

Aquaculture is the fastest-growing food production sector and has become a cornerstone of national development plans [1], with more than half of all aquatic food destined for human consumption now produced on farms [2]. As capture fisheries production continues to decline, meeting the increasing demand for animal protein falls upon this sector.

Among cultured species, tilapia (*Oreochromis* spp.) stands out as one of the most significant ones [3], contributing approximately 40% of global farmed fish production [1]. This prominence is attributed to the remarkable biological characteristics of tilapia, including high adaptability, rapid growth rates, and tolerance to a wide range of environmental conditions [4].

To maximize economic returns, commercial farms commonly rely on the culture of monosex male hybrid tilapia, produced by crossing blue tilapia males (*Oreochromis aureus*) with Nile tilapia females (*O. niloticus*). These fish exhibit superior growth rates and size uniformity, while also preventing unwanted reproduction within rearing units [4, 5]. Furthermore, monosex culture technology allows for extended rearing periods for fingerlings and improved feed conversion ratios, with the flexibility to use fingerlings at different age stages [6].

Tilapia farming systems are broadly categorized into three main production systems, varying in stocking density: extensive (low-density, relying on natural food), semi-intensive (moderate densities with supplemental feeds), and intensive (high densities, continuous aeration, and complete feeding). Stocking densities vary widely in practice; in semi-intensive earthen ponds, densities range around 5 fish/m³, whereas in intensive systems (e.g., cages and concrete ponds), densities range from 160 to 400 fish/m³ and may reach up to 500 fish/m³ when adequate aeration and balanced nutrition are ensured [7].

Determining the appropriate stocking density is one of the most critical management decisions, directly influencing productivity and profitability [8, 9]. While higher densities enable greater total production per unit area, they may lead to increased competition for food and space, deterioration of water quality, affecting growth rates, reducing feed conversion efficiency, and affecting flesh quality [10]. Conversely, lower densities contribute to a more balanced rearing environment [11].

Alongside stocking density, the aquaculture sector faces a growing challenge of freshwater scarcity, driving a shift toward alternative water sources such as brackish water (10–15‰) or treated agricultural drainage water [12]. Although tilapia are classified as euryhaline species, exposure to sub-optimal salinity concentrations (e.g., 10‰) imposes osmotic stress, which diverts metabolic energy toward osmoregulation at the expense of growth [13].

Despite the abundance of studies examining the individual effects of stocking density and salinity on tilapia performance, there remains a notable scarcity of research on the interactive effects of these two variables, particularly at a specific salinity level (10‰). Therefore, elucidating how these environmental stressors interact is essential for optimizing production and enhancing its efficiency in regions suffering from freshwater shortages. Thus, investigating the effect of stocking density on productive performance and chemical composition of monosex male tilapia under low-salinity conditions is of utmost importance.

Research Aims and Importance:

This study aims to:

- Evaluate the growth performance, feed conversion efficiency, chemical composition, and survival rate of monosex tilapia fingerlings reared under low-salinity stress.
- Investigate the effect of different stocking densities on the above-mentioned parameters, and determine the optimal stocking density.

Materials and Methods:

Acclimation of Fingerlings:

Monosex tilapia fingerlings were obtained from the fish farm of Al-Sin River/Banyas/Syria. They were transported in plastic bags, filled with water and saturated with oxygen [14], to the Laboratory of Zoology, Faculty of Science, Latakia University, in August 2023.

Fingerlings were placed in glass tanks, each containing 35 liters of freshwater and equipped with air pumps. They were left unfed for 48 hours for recovery from transport stress.

Acclimation to a low salinity of 10‰ was carried out over a period of 30 days (August 2–31, 2023); during that period, salinity was gradually increased from ≤ 0.5 ‰ (freshwater) to 10‰ by adding seawater. Throughout this period, the fingerlings were fed a diet containing 32.5% crude protein (Table 1). Behavior and mortality rates were monitored daily; water quality parameters were regularly checked with periodic water exchanges to prevent the accumulation of toxic metabolic wastes.

Table (1): Composition of the experimental diet per 100 g of feed

Ingredient	Amount (g)
Soybean meal	45
Cottonseed meal	10
Poultry by-product meal	18
Wheat bran	15
Yellow corn meal	7
Dicalcium phosphate	1.5
Choline chloride	0.5
Methionine	0.5
Mineral premix	1
Vitamin premix	1.5
Total	100

Feeding:

Following the acclimation period, initial body weights of the fingerlings were recorded using a sensitive digital balance (OPTICA-Italy). Fingerlings were randomly distributed, with similar sizes, into nine glass ponds, representing three treatments with three replicates each, as follows:

- Treatment 1 (D1 – Low density): 5 fish/aquarium (143 fish/m³).
- Treatment 2 (D2 – Medium density): 10 fish/aquarium (286 fish/m³).
- Treatment 3 (D3 – High density): 20 fish/aquarium (571 fish/m³).

The rearing period lasted 73 days (September 1 to November 12, 2023). During this period, fish were fed twice daily (9:00 AM and 3:00 PM) with the experimental diet (Table 1) having a pellet size of 3–4 mm, at a rate of 4–5% of their body weight per day. The amount of feed offered to each aquarium was recorded daily.

Water quality was maintained within optimal ranges; solid wastes were removed daily using a hand net, partial water exchanges (40–50% of total volume) were performed daily, and complete water renewal was conducted weekly. Hydrological parameters were measured daily using a WTW-Multi34DI.

At the end of the rearing period, fish were fasted for 24 h. Prior to final weighing, fish were allowed to evacuate their guts. They were then harvested, and final weights were recorded (three measurements per sample to ensure accuracy) for all fish in each pond individually. The following parameters were calculated:

- Average Daily Gain (ADG) = (Final weight – Initial weight) ÷ Rearing duration (days).
- Feed Conversion Ratio (FCR) = Dry feed offered (g) ÷ Wet weight gain (g).
- Survival Rate (SR %) = (Final number of fish ÷ Initial number of fish) × 100.

Proximate Chemical Analysis of Body Tissues:

Whole-body fish samples of each replicate were oven-dried at 60°C for 24 hours until a constant weight was achieved, then ground into a fine powder. Chemical analysis was performed according to the standard methods [15]:

- Crude Protein: Determined by the Micro-Kjeldahl method using a Büchi B-324 distillation unit, and calculated by multiplying total nitrogen by the conversion factor of 6.25.
- Crude Lipid: Extracted by soaking in hexane (C₆H₁₄) for 24 hours, followed by filtration and evaporation of the solvent in a water bath at 70°C, after which the extracted oil weight was recorded.
- Ash: Determined by incineration of the samples in a muffle furnace at 600°C for 5 hours.

Statistical Analysis:

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Significance was set at $P < 0.05$. Results are presented as means ± standard deviation.

Results and Discussion:

Effect of Stocking Density on Water Quality:

Good pond management, reflected in adequate aeration and regular water exchange, played a significant role in preventing water quality from deteriorating to unacceptable levels. Despite the high stocking densities, measurements of hydrological parameters (Table 2) showed that all values, particularly temperature and salinity, remained within the optimal ranges for tilapia growth, as reported by Popma and Lovshin (1996) [17].

Table (2): Hydrological Factors of Water During Rearing Period

Diets	D1 (143 fish/m ³)	D2 (286 fish/m ³)	D3 (571 fish/m ³)
Temperature (°C)	25.05±1.07	25.05±1.07	25.05±1.07
Dissolved Oxygen (mg/L)	5.53±0.47	5.23±0.49	4.45±0.32
Salinity(‰)	10.39±0.43	10.39±0.43	10.39±0.43
pH	7.9±0.11	7.8±0.10	6.5±0.20

Nevertheless, pH and dissolved oxygen values recorded from the treatment D3 approached the lower permissible limits. However, they did not reach the critical levels that could cause mortality or severe growth inhibition [16, 17]. This confirms that the observed differences in fish performance among treatments are primarily attributable to the effect of stocking density per se, rather than to a sharp deterioration in water quality.

In the study of Roldi *et al.* (2025), water quality parameters during the rearing period ranged between 3.83 and 5.35 mg/L for dissolved oxygen, and between 7.44 and 7.6 for pH, with all values decreasing as stocking density increased [18].

Effect of Stocking Density on Survival Rate:

Survival rate was the highest when reducing densities to 5 and 10 fish/35 L compared to increasing them to 20 fish/35 L, with survival rates reaching 100% for both D1 and D2 treatments, while only 90% survival was recorded at D3 (Table 3). This finding is consistent with the results of Hassan *et al.* (2025), who reported that both survival and growth rates exhibited an inverse relationship with stocking density [23]. Furthermore, the findings of El-Dahhar *et al.* (2021) indicated that predation behavior increases with higher fish densities, leading to reduced survival rates [11].

Table (3): Weight Gain, Daily Growth Rate, Feed Conversion Ratio, and Survival Rate of Experimental Fish

Density	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Feed Amount (g)	Daily Growth Rate	Feed Conversion Ratio	Survival Rate
D1 (143 fish/m ³)	27.66 1.2±	38.37 2.3±	10.71	12.34	0.147	1.15	%100
D2 (286 fish/m ³)	26.45 2.2±	41.02 2.05±	14.57	19.16	0.199	1.31	%100
D3 (571 fish/m ³)	23.74 1.7±	29.06 0.95±	5.32	16.52	0.073	3.10	%90

Effect of Stocking Density on Growth Performance and Feed Utilization:

The results demonstrated a significant superiority ($P < 0.05$) of treatments D1 and D2 over D3 in terms of average daily gain and feed conversion efficiency. The highest numerical daily gain was recorded in D2 (0.199 g/day), while D1 (0.147 g/day) and D2 did not differ significantly ($P > 0.05$). The best feed conversion ratio was observed in D1 (1.15), followed by D2 (1.31), whereas D3 recorded the lowest growth rate (0.073 g/day) and the highest FCR (3.10). These results suggest that low and medium densities provided more favorable conditions for fish growth, where competition for food and space was reduced, thereby lowering overall stress levels.

These results are in agreement with several previous studies, in which increased stocking density of tilapia was associated with reduced growth and elevated feed conversion ratio. This is attributed to the intensified aggressive behavior, energy expenditure for stress adaptation [18], and elevated cortisol levels that suppress appetite and stimulate basal metabolism [19]. Similarly, the study of Ammar (2009) yielded the same outcome, where mean weight gain, daily and specific growth rates, and feed conversion ratio of tilapia were significantly affected by stocking density [20].

In the study of Ani *et al.* (2022), specific growth rate decreased with increasing stocking density, while feed conversion ratio increased with density, and low-density aquaculture systems performed better than those stocked at 300 or 450 fish/m³ [18].

In the study of Barcellos *et al.* (2001), one possible explanation for this result was tank size, wherein each fish establishes a specific position in a corner of the tank, thereby preventing fighting or aggressive behavior, which in turn reduces stress and energy expenditure, consequently enhancing growth rate and improving feed conversion ratio [21].

In the study of Hassan *et al.* (2025), the optimal stocking density for Nile tilapia in sustainable cage culture systems ranged between 25 and 35 fish/m³, and the results indicated that Nile tilapia growth decreases with increasing stocking rate, with the best

growth performance and survival rate were recorded at low stocking density; the lowest value of the feed conversion ratio (FCR) was observed at lower densities ($P < 0.05$), and the profit index was highest at low density [22].

In the present study, the relative salinity stress (10‰) appears to have imposed an additional load on the fish's physiological systems. At low density, it is presumed that fish were able to allocate a greater proportion of their consumed energy to growth after meeting the osmoregulatory demands. At high density (D3), however, the stress may have been exacerbated by crowding, leading to increased energy depletion (due to the combined demands of salinity adaptation and social stress) and markedly inhibited growth, which was reflected in an elevated FCR of 3.10 in D3 compared to 1.15 at low density.

One-way ANOVA revealed significant differences in average daily gain among the three treatments ($P < 0.01$). The results also showed a significant superiority of treatment D2 (0.199 g/day) compared to D3 (0.073 g/day), while D1 and D2 did not differ significantly ($P > 0.05$). Regarding feed conversion ratio, the differences were also significant ($P < 0.001$), with D3 recording the highest value (3.10) compared to D1 (1.15) and D2 (1.31).

Effect of Stocking Density on Proximate Body Composition:

Proximate chemical analysis of whole-body composition (Table 4) revealed a clear effect of stocking density on the chemical composition of monosex tilapia reared under salinity stress conditions. Crude protein content decreased from 59.73% at low density (D1) to 52.93% at high density (D3). Similarly, crude lipid content declined from 6.63% to 5.07%, while ash content increased from 20.43% to 23.35%.

The reduction in protein content is attributed to an elevated metabolic rate under dual stress conditions (low salinity and crowding), wherein fish deplete their protein reserves to meet the heightened energy demands rather than directing them toward growth. The decrease in lipid content reflects their utilization as a rapid energy source for stress adaptation, or alternatively, indicates poor feed conversion efficiency and reduced energy storage.

The elevated ash content at high density suggests alterations in bone tissue and mineral composition resulting from disturbances in osmotic and hormonal balance [18]. The higher carbohydrate and fiber content recorded in D3 (18.65%) compared to D1 (13.21%) is attributed to the reduction in other major components, which proportionally increased their relative percentages in the overall composition.

Table (4): Chemical Composition of Experimental Fish (g/100g)

Density	Protein	Fat	Ash	Other components*
D1 (143 fish/m ³)	1.38± 59.73	0.14± 6.63	0.06± 20.43	%13.21~
D2 (286 fish/m ³)	1.80 ± 61.76	0.01± 6.18	0.01± 18.76	%13.30~
D3 (571 fish/m ³)	0.69± 52.93	1.88 ±5.07	0.17± 23.35	%18.65~

*Other components include carbohydrates, fiber, and various inorganic materials.

In agreement with those of Roldi *et al.* (2025), who reported that Nile tilapia reared in high-density tanks exhibited reduced lipid metabolism efficiency. Conversely, fish from low-density tanks contained higher concentrations of polyunsaturated fatty acids, confirming the influence of density on nutritional quality [18]. These results demonstrate that low density (143 fish/m³) is the most suitable for achieving the highest flesh quality under 10‰ salinity conditions, as it yielded the highest protein and lipid contents, along

with the lowest ash and carbohydrate levels. This supports the recommendation to adopt this density in intensive culture systems in regions suffering from freshwater scarcity.

Study Limitations:

The authors acknowledge the following limitations:

- Initial body weights varied among treatments (D1=27.66g, D2=26.45g, D3=23.74g), which may have influenced absolute weight gain.
- The small experimental units (35-L aquaria) represent a laboratory-scale model; field application requires caution.
- Proximate composition was analyzed only at the end of the experiment; initial homogeneity was assumed but not verified.

Conclusions and Recommendations:

- Increased stocking density resulted in the deterioration of growth performance, feed conversion efficiency, and survival rate of monosex tilapia fingerlings reared under low-salinity (10‰).
- High density negatively affected flesh quality, reducing both protein and lipid contents in whole-body composition.
- Low density (143 fish/m³) and medium density (286 fish/m³) yielded comparable growth rates ($P > 0.05$), but the low density is recommended for achieving the best feed conversion ratio and maintaining high flesh quality.

This study recommends the following:

- Application of the findings of this study on a field-scale aquaculture level (in concrete or earthen ponds) to validate the results under production conditions more closely resembling natural environments.
- Conducting future studies to evaluate the effect of the same stocking densities under other salinity levels (e.g., 5 and 15‰).
- Investigating physiological and immunological indicators (such as blood cortisol and glucose levels) to better understand the mechanisms of fish response to stress levels resulting from different treatments.

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