

Original Research Articles

Effects of stocking size, density, and feeding frequency on growth performance of *Centropristis striata* in a recirculating aquaculture system

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Keywords: Stocking size, Stocking density, Feeding frequency, Growth performance, Orthogonal experiment, *Centropristis striata*

<https://doi.org/10.46989/001c.167720>

Israeli Journal of Aquaculture - Bamidgheh

Vol. 78, Issue 3, 2026

Centropristis striata is a valuable marine fish native to the Atlantic coast of the United States and Mexico. In recent years, it has become an excellent species for promotion and breeding in the eastern and southeastern coastal areas of China. In order to explore the effects of different stocking size, stocking density and feeding frequency on the growth performance of *C. striata*, and to screen the optimal combination of culture parameters, and to provide a theoretical basis for aquaculture production practice, L₉ (3³) experimental design was used to set up three factors and three levels of stocking size (200 g tail⁻¹, 400 g tail⁻¹, 600 g tail⁻¹), stocking density (30 kg m⁻³, 40 kg m⁻³, 50 kg m⁻³) and feeding frequency (1,2,3 times day⁻¹). A total of 9 experimental groups were set up, with 3 replicates per group. The test period was 8 weeks. The specific growth rate (SGR) and survival rate of each group were measured, and the results were analyzed using range and variance analyses. The results showed that the effects of the three factors on the survival rate were not significant, and the survival rate in each group was higher than 95%. Culture size and stocking density had a significant effect on the specific growth rate. As stocking size increased, the specific growth rate gradually decreased. With the increase of stocking density, the specific growth rate increased gradually, and the specific growth rate was the highest (0.45%) in the combination of 200 g tail⁻¹, 50 kg m⁻³ and 3 times day⁻¹. The range analysis showed that the primary and secondary order affecting the specific growth rate was: stocking size (R = 0.29) > breeding density (R = 0.10) > feeding frequency (R = 0.05). Based on the comprehensive growth performance, the optimal combination with the goal of rapid weight gain was 200 g tail⁻¹, 50 kg m⁻³, feeding 3 times day⁻¹; the optimal combination with high survival rate was 400 g tail⁻¹, 30 kg m⁻³, feeding twice a day. This study can serve as a reference for parameter optimization in the industrial breeding of *C. striata*.

1. INTRODUCTION

Centropristis striata belongs to Serranidae, Perciformes, and Actinopterygii. It is mainly distributed in the United States and Mexico along the Atlantic coast and belongs to a kind of marine fish with high economic value.¹ In recent years, fish have been paid more and more attention in the field of mariculture production and scientific research in China because of their tender meat, good commodity acceptance,

and certain adaptability to the environment. *C. striata* has a wide range of temperatures and salinities. The suitable temperature for its growth is mostly between 17°C and 25°C, and the suitable salinity for its growth is about 20‰ ~ 30‰.² Following the introduction of *C. striata* into China, coastal regions including Fujian, Zhejiang, and Shanghai have conducted experimental and production trials in recent years, covering the full production spectrum from seed breeding, pond culture, and cage culture to industrial recirculating aquaculture.³ In recent years, following the in-

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roduction of *C. striata* into China, coastal regions have conducted aquaculture trials using various culture systems, including seed rearing, pond culture, cage culture, and factory recirculating aquaculture. In practical industrial farming, initial stocking size, stocking density, and feeding frequency are three key controllable operational parameters that directly determine growth performance, production efficiency, and farming profitability. However, the combined effects and the optimal matching scheme among these three factors in recirculating aquaculture of *C. striata* remain poorly defined.

Stocking size is one of the important factors affecting the growth rate, breeding cycle and production arrangement of *Dicentrarchus labrax*⁴ and *C. striata*.⁵ From the general law of fish culture, individuals at different growth stages have differences in metabolic level, feeding ability, nutritional needs and environmental adaptability. Therefore, fish with different initial specifications often show different growth effects under the same culture conditions.⁵ Small fish were in a rapid growth phase, with higher relative and specific growth rates. With the increase of fish size, the growth rate will gradually slow down, and the feed conversion efficiency may also change.⁶ Watanabe et al.⁷ pointed out in the study of *C. striata* culture that the fish has good culture potential, but the growth rate, feed utilization, and individual differences in different size stages will affect the breeding effect. Pan et al.⁸ also found that reasonable control of seedling specifications and hierarchical management were conducive to improving the culture survival rate and reducing differences in individual size. Zhao et al.⁹ found in the study of grouper nutrition that there were differences in feed protein demand across grouper species.

Stocking density is a key parameter to be controlled in the culture of *Centropristis striata*.¹⁰ Appropriately increasing stocking density can increase yield per unit of water body. However, when the density is too high, the activity space of the fish body is reduced, the feeding competition between individuals is enhanced, the consumption of dissolved oxygen in water is accelerated, and harmful substances such as ammonia, nitrogen, and nitrite are more likely to accumulate.¹¹ These changes may cause stress responses in fish, thus affecting their feeding, growth, immune function, and health status. Mugwany et al.¹² documented in their review of recirculating aquaculture that stocking density affects the behavior, growth performance, and immune status of fish, but the suitable density varies across fish species, growth stages, and system conditions. Carroll et al.¹³ studied the growth, feed utilization, and anti-crowding ability of juvenile *C. striata* under high-density conditions. The results showed that the fish could maintain good growth performance at higher stocking density.

Feeding frequency is one of the important factors affecting the feeding, growth and feed utilization of *Centropristis striata*. Reasonable feeding frequency can enable the fish to obtain a more stable nutrient supply in a day, which is conducive to maintaining normal feeding rhythm and improving feed utilization efficiency.¹⁴ If the feeding frequency is too low, fish growth may be affected by insufficient feed-

ing opportunities. If the feeding is too frequent, it is easy to cause the increase of residual feed and the waste of feed, and aggravate the pollution load of water body, which has adverse effects on the water circulation system. In the study of *Epinephelus lanceolatus*, Qiu et al.¹⁵ found that feeding frequency could affect fish growth, feeding and immune enzyme activity. Fava et al.¹⁶ found that the weight gain of fish fed 5 and 6 times a day was significantly higher than that of 4 times ($p < 0.05$), but there was no significant difference with 7, 8, and 9 times groups. Gilannejad et al.¹⁷ studied the effects of different daytime feeding frequencies on the circadian rhythm of digestive function in the gastrointestinal tract of juvenile *Sparus aurata*. Feeding frequency had little effect on the circadian phase of biological clock genes, and the expression of most digestive enzyme precursor genes was not significantly affected by it. In actual culture production, appropriate feeding schemes should be formulated for different growth stages to account for growth rate and feed utilization efficiency.

There has been some research on recirculating aquaculture of *C. striata*, but studies on changes in growth performance across different stocking sizes, stocking densities, and combinations of feeding frequencies remain insufficient. Especially in production practice, optimizing a single factor often fails to fully reflect the actual situation in the culture process. Stocking size will affect the growth stage and nutritional requirements of fish. Stocking density will affect water-quality loads and individual competition. Feeding frequency is related to feeding rhythm, feed utilization and water stability. In this work, *C. striata* was used as the research object, and the experiment was conducted under recirculating aquaculture conditions. The three-factor, three-level orthogonal experimental design was used to compare the effects of combinations of stocking size, stocking density, and feeding frequency on survival rate, specific growth rate, and other indicators. The degree of action of various factors on growth performance was analyzed, and the most suitable combination of culture parameters under the experimental conditions was selected. The results of this study can provide basic data for optimizing the culture parameters of *C. striata* and serve as a reference for subsequent large-scale culture.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL MATERIALS

In this experiment, three kinds of *C. striatas* with good health were taken from the Ganyu Base of the East China Sea Fisheries Research Institute, which were 200 g per fish, 400 g per fish, and 600 g per fish. The daily feeding feed is the high-grade fish high-quality compound feed floating type L5 (crude protein content greater than 50.0%, crude fat content greater than 10.0%, crude fiber less than 8.0%, crude ash less than 17.0%, lysine greater than 2.4%, moisture less than 12.0%) produced by Qingdao Sai Green Marine Biological Feed Co., Ltd.

Table 1. Orthogonal test design

Group	Stocking size (g tail ⁻¹)	Stocking density (kg m ⁻³)	Feeding frequency (times day ⁻¹)
1	200	30	1
2	200	40	2
3	200	50	3
4	400	30	2
5	400	40	3
6	400	50	1
7	600	30	3
8	600	40	1
9	600	50	2

2.2. EXPERIMENTAL DESIGN

Three factors and three levels orthogonal experimental design $L_9 (3^3)$ was used to investigate the three factors of stocking size, stocking density and feeding frequency (Table 1).

Each group was fed daily according to the experimental design requirements, and the feeding amount, residual feed, and death were recorded in time. The number of vibrios in the water was detected every Monday, and the concentrations of ammonia nitrogen, nitrite and hydrogen sulfide were measured every 3 days to ensure that the grouper was always in a suitable environment. The experimental period was 8 weeks. Each group had three replicates (biological replicates). The total number of cages was 27, and the effective water body of each cage was 0.54 m³ (0.9 m * 1.2 m * 0.6 m). The stocking size are divided into three specifications, which are 200 g tail⁻¹, 400 g tail⁻¹, and 600 g tail⁻¹. The stocking density is calculated according to the weight of unit water body, which is 30 kg m⁻³, 40 kg m⁻³ and 50 kg m⁻³, respectively. Feeding frequency is once a day, twice a day, or three times a day. The total daily feeding amount is calculated as 0.8% of the growing weight, and the feeding time was once a day (8: 30 a.m., 100%), twice a day (8: 30 a.m., 50%; 8: 30 p.m., 50%), three times a day, 8: 30 a.m., 50%, 2: 00 p.m., 20%, 8: 30 p.m.). According to the feeding situation, or increase or decrease, each increase or decrease to 5% or 10%. Fish were fasted once a week.

2.3. DETERMINATION AND METHOD OF GROWTH INDEX

At the beginning and end of the experiment, the number and weight of all fish were measured, and the feeding was stopped after 24 hours. The growth index of the experimental fish was calculated according to the following formula.

$$SGR = (\ln W_t - \ln W_0) / T \times 100$$

$$SR = Q_t / Q_0 \times 100\%$$

In the formula: *SGR* is the specific growth rate; W_t is the average weight at the end of the experiment; W_0 is the average tail weight at the beginning of the experiments; T is the experimental days; *SR* is the survival rate; Q_t represents the total number of fish at the end of the experiment; Q_0

represents the total number of fish at the beginning of the experiment.

2.4. DATA PROCESSING AND ANALYSIS

All experimental data were processed by IBM SPSS Statistics 22 for range analysis, three-way analysis of variance (Three-Way ANOVA), and Duncan's method for multiple comparisons. $p < 0.05$ was considered significant.

3. RESULTS

3.1. THE SURVIVAL RATE AND GROWTH PERFORMANCE OF THREE SPECIFICATIONS OF *CENTROPRISTIS STRIATA*

The survival rate of *C. striata* across all treatment groups remained high. The survival rate of the combination of 400 g stocking size, 40 kg m⁻³ density and feeding frequency of 3 times day⁻¹ reached 100%. Multiple comparisons of survival rates showed no significant differences among treatment groups ($p > 0.05$).

It can be seen from Table 2 that the growth of groups 1, 2, and 3 with a 200 g stocking size was the fastest, and the specific growth rate (SGR) ranged from 0.26% to 0.45%. Under the treatment of 50 kg m⁻³ density and 3 times day⁻¹ feeding, the specific growth rate reached the highest of 0.45%, and the absolute weight gain also reached 52.64 g, which was the highest in all groups.

For 400 g grouper, the growth rate slowed down, and the specific growth rate ranged from 0.12% to 0.19%. The 600 g grouper, the largest size group, grew the slowest, with a specific growth rate of only 0.02% ~ 0.10%. From the perspective of Stocking size, SGR decreased significantly with the increase of stocking size. The average SGR of each frequency of 200 g was the highest, followed by 400 g and 600 g. From the perspective of feeding frequency, the average SGR (0.21) of 3 times day⁻¹ was the highest, 2 times per day was the lowest (0.16), and 1 time per day was the middle (0.19). The SGR was the highest (0.45) when high-density culture (50 kg m⁻³) was combined with high-frequency feeding (3 times day⁻¹). However, when high density was combined with low frequency (1 time day⁻¹), SGR was lower (0.11).

Table 2. Survival rate and growth performance of *Centropristis striata* under different factors and different levels

Group	Stocking size (g tail ⁻¹)	Stocking density (kg m ⁻³)	Feeding frequency (times day ⁻¹)	Survival rate (%)	Initial weight (g)	Final weight (g)	Specific growth rate (%)
1	200	30	1	96.25±2.50 ^a	197.93±2.99	228.58±11.35	0.26±0.06
2	200	40	2	97.67±2.08 ^a	193.33±1.53	232.97±5.03	0.33±0.04
3	200	50	3	96.41±2.91 ^a	181.53±3.67	234.17±3.36	0.45±0.02
4	400	30	2	95.00±0.00 ^a	409.20±2.59	455.26±2.63	0.19±0.01
5	400	40	3	100.00±0.00 ^a	415.33±9.71	447.60±19.06	0.13±0.03
6	400	50	1	98.89±0.01 ^a	411.40±9.18	440.78±13.93	0.12±0.05
7	600	30	3	95.06±2.14 ^a	608.67±4.68	646.26±26.58	0.11±0.06
8	600	40	1	99.02±1.70 ^a	604.43±7.35	612.90±3.22	0.02±0.01
9	600	50	2	95.83±7.22 ^a	613.36±12.75	631.39±22.22	0.05±0.05

Table 3. Orthogonal experiment variance analysis

Source of variation	SS	df	MS	F	p
Intercept	254701.338	1	254701.338	31735.805	0.000**
Stocking size (g tail ⁻¹)	9.551	2	4.775	0.595	0.561
Stocking density (kg m ⁻³)	16.023	2	8.011	0.998	0.386
Feeding frequency (times day ⁻¹)	53.905	2	26.953	3.358	0.055
Residual	160.514	20	8.026		

3.2. ANALYSIS OF VARIANCE AND RANGE ANALYSIS OF DIFFERENT FACTORS AND DIFFERENT LEVELS ON THE SURVIVAL RATE OF *CENTROPRISTIS STRIATA*

According to Table 3, in this experiment, the Stocking size and stocking density did not show a significant difference, so the Stocking size and stocking density had no significant effect on the survival rate. The feeding frequency also did not show significant ($p = 0.055$), but it was close to the significant level of 0.05, which was marginally significant, indicating that the feeding frequency had a certain influence trend on the survival rate, but did not reach a statistically significant difference.

From the range analysis of the specific survival rate (SGR) of *C. striata* under various experimental conditions, Table 4 shows that R-feeding frequency (3.46) > R-stocking density (1.89) > R-stocking size (1.32). It can be concluded that feeding frequency is the main influencing factor, followed by stocking density and, finally, stocking size. In the specification factor, $k_2 > k_1 > k_3$, that is, in the specification factor, the 2 level 400 g *Centropristis striata* is the optimal level; in the stocking density factor, $k_1 > k_3 > k_2$, that is, the level of 30 kg/m³ in the stocking density factor is the optimal level; in the feeding frequency factor, $k_2 > k_3 > k_1$, that is, the feeding frequency factor in the 2 level 2 times day⁻¹ is the optimal level. Therefore, the best combination condition to improve the survival rate is 400 g tail⁻¹-30 kg m⁻³-2 times day⁻¹.

3.3. ANALYSIS OF VARIANCE AND RANGE ANALYSIS OF DIFFERENT FACTORS AND DIFFERENT LEVELS ON THE SPECIFIC GROWTH RATE OF *CENTROPRISTIS STRIATA*

From Table 5, the breeding specifications and stocking density showed significant differences, so they will have different relationships with the survival rate. There was no significant difference in feeding frequency ($p = 0.233$), so feeding frequency did not have a differential relationship with specific growth rate.

From the range analysis of the specific growth rate of *Centropristis striata* under various experimental conditions, Table 6 shows that R-stocking size (0.29) > R-stocking density (0.10) > R-feeding frequency (0.05). Therefore, stocking size are the main influencing factors, followed by stocking density, and finally feeding frequency. By comparing the K_{avg} values of different levels of each factor, the best level of each factor was determined. It can be seen that the three best levels are 200 g tail⁻¹ of stocking size, 50 kg m⁻³ of stocking density, and 3 times day⁻¹ of feeding frequency.

4. DISCUSSIONS

4.1. EFFECTS OF DIFFERENT FACTORS AND LEVELS ON THE SURVIVAL RATE AND SPECIFIC GROWTH RATE OF *CENTROPRISTIS STRIATA*

In this study, a three-factor, three-level orthogonal experimental design was used to systematically explore the ef-

Table 4. Range analysis of orthogonal test

Item	Level	Stocking size (g tail ⁻¹)	Stocking density (kg m ⁻³)	Feeding frequency (times day ⁻¹)
K	1	870.98	882.48	858.94
	2	881.67	865.50	890.06
	3	869.74	874.42	873.40
K _{avg}	1	96.78	98.05	95.44
	2	97.96	96.17	98.90
	3	96.64	97.16	97.04
Optimal level		2	1	2
R		1.32	1.89	3.46
Number of levels		3	3	3
Replicates per level r		9.0	9.0	9.0

Table 5. Three-factor analysis of variance of specific growth rate

Source of variation	SS	df	MS	F	p
Intercept	0.932	1	0.932	304.511	0.000**
Stocking size (g tail ⁻¹)	0.389	2	0.194	63.473	0.000**
Stocking density (kg m ⁻³)	0.043	2	0.021	6.960	0.005**
Feeding frequency (times day ⁻¹)	0.010	2	0.005	1.570	0.233
Residual	0.061	20	0.003		

Table 6. Range analysis of specific growth rate

Item	Level	Stocking size (g tail ⁻¹)	Stocking density (kg m ⁻³)	Feeding frequency (times day ⁻¹)
K	1	3.13	1.21	1.66
	2	1.34	1.73	1.47
	3	0.55	2.08	1.89
K _{avg}	1	0.35	0.13	0.18
	2	0.15	0.19	0.16
	3	0.06	0.23	0.21
Optimal level		1	3	3
R		0.29	0.10	0.05
Number of levels		3	3	3
Replicates per level r		9.0	9.0	9.0

fects of stocking size, density and feeding frequency on the survival rate and specific growth rate of *C. striata* in recirculating aquaculture. The survival rate of all treatment groups exceeded 95%, with no significant inter-group differences. Even at the high stocking density of 50 kg m⁻³, *C. striata* maintained a very high survival rate, which is consistent with the conclusion of Carroll et al.¹³ noted that juvenile *C. striata* can sustain normal growth and survival under high-density culture and exhibits strong high-density tolerance.

Range analysis was applied to rank the relative influence weight of each factor, as the R value reflects the magnitude of each factor's effect on the target indicator.¹⁸ The results

showed divergent dominant factors for the two indicators. For the survival rate, feeding frequency had the highest influence on weight, followed by stocking density and stocking size, with the optimal combination being 400 g tail⁻¹ stocking size, 30 kg m⁻³ density and 2 times day⁻¹ feeding. For SGR, stocking size was the dominant factor, followed by stocking density and feeding frequency, with the optimal combination being 200 g tail⁻¹ stocking size, 50 kg m⁻³ density and 3 times day⁻¹ feeding.

The marginal significance of feeding frequency on survival ($p = 0.055$), despite its top-ranking in the range analysis, can be explained by both statistical and biological mechanisms. First, a ceiling effect was present as overall

survival remained above 95% across all treatments; this high baseline compressed the magnitude of between-group differences and limited statistical power to detect formal significance. Second, the recirculating system's stable water-quality regulation capacity buffered the potential negative impacts of residual feed and metabolic waste resulting from inappropriate feeding frequency, further reducing between-group variation in survival. Feeding frequency affects survival indirectly by modulating feeding equity and digestive load rather than imposing direct lethal stress, so its effect is relatively mild and fails to reach the conventional significance threshold under favorable culture conditions.

The divergence in dominant factors between survival and SGR is attributed to the inherent properties of the two indicators. Survival is a threshold trait primarily driven by short-term physiological stability and health status in non-stressful environments, so factors related to feeding behavior show the strongest relative influence. In contrast, SGR is a cumulative growth trait predominantly determined by the endogenous growth stage of fish, whereas exogenous factors such as density and feeding frequency play only a secondary regulatory role. The two distinct optimal combinations correspond to survival-oriented and growth-oriented production strategies, respectively, providing flexible references for different farming objectives.

4.2. EFFECTS OF FEEDING FREQUENCY ON GROWTH PERFORMANCE OF *CENTROPRISTIS STRIATA*

Different feeding frequency gradients affect the growth performance of fish. Marimuthu et al.¹⁹ conducted an experiment on the feeding frequency of African catfish fry and found that survival rates were higher when fed once a day or twice a day, reaching $96.67\% \pm 5.77\%$ and $86.67\% \pm 5.77\%$, respectively. However, the survival rates of each experimental group were not statistically significant. Li et al.²⁰ clearly pointed out that feeding twice a day (7: 30, 16: 30) was optimal, and over-frequency inhibited weight gain. Xu et al.²¹ studied the effects of feeding frequency on the growth, intestinal health, and metabolism of *H. wyckioides* cultured in a land-based circular tank, and found that feeding frequency three times a day improved the growth performance of *H. wyckioides* in aquaculture by increasing the abundance of beneficial clostridium and trichomonas, activating multiple immune pathways, and enhancing amino acid metabolism. Wu et al.²² found that as feeding frequency increased, body lipid content increased, while water content decreased. Under the conditions of this experiment, the survival rate of each feeding frequency group was above 95 %, and the effect of feeding frequency on the survival rate of *Centropristis striata* was not statistically significant ($p > 0.05$). Feeding frequency had a significant effect on the growth of small-sized *C. striata*, and the specific growth rate of small-sized fish increased with the increase of feeding frequency. The stomach capacity of juvenile fish is small, and the single food intake is limited. Multiple feeding can effectively improve its food intake and meet the nutritional needs of rapid growth.²³

4.3. EFFECTS OF STOCKING SIZE ON GROWTH PERFORMANCE OF *C. STRIATA*

Stocking size is one of the key internal factors affecting the growth performance of fish. The range analysis of orthogonal experiments in this study showed that the small size of *C. striata* was in the rapid growth period and had greater growth potential, while the large size individuals had more advantages in stress resistance and survival stability.²⁴ Stocking size may also regulate growth performance by affecting feeding behavior, metabolic efficiency, and energy distribution mechanisms. Small-sized individuals usually have higher relative metabolic rates, and energy is used more for protein synthesis and body tissue growth, rather than fat storage.²⁵ Sogard et al.²⁶ examined the changes of lipid deposition in juvenile fish *Anoplopoma fimbria* and found that compared with large juvenile fish, small juvenile fish allocated more energy for growth and less energy for lipid storage. With increasing size, the proportion of maintenance metabolism in fish energy demand increased, and the relative growth rate naturally decreased. At the same time, large individuals exhibit greater survival stability in the face of water quality fluctuations and operational stress due to their greater environmental adaptability and stress resistance.

4.4. EFFECTS OF STOCKING DENSITY ON GROWTH PERFORMANCE OF *C. STRIATA*

Stocking density is an important factor affecting water productivity. With the increase of stocking density, the yield per unit water body also increases. Meanwhile, elevated density accelerates dissolved oxygen consumption, carbon dioxide emission, and ammonia nitrogen accumulation, which may deteriorate water quality and further affect feeding, growth, energy metabolism, and behavior of cultured organisms.²⁶ Hassan et al.²⁷ have shown that both stocking density and dietary protein level can affect the growth and feed conversion rate of fish, but have no significant impact on survival rate within suitable ranges. Prihadi et al.²⁸ conducted a study on *Oxyeleotris marmorata* and found that under different stocking densities (50, 100, 150 individuals m^{-3}), the survival rates were 99%, 100%, and 100%, respectively. Within the scope of the study, the increase of stocking density did not significantly affect the survival rate of fish, and the survival rate was maintained at a very high level, indicating that fish adapted well under experimental conditions and had no obvious density stress effect. In this study, as stocking density increased, the K_{avg} value of the specific growth rate gradually increased from 0.13 to 0.23, indicating a significant upward trend, with the optimal level at 50 $kg\ m^{-3}$. In the density range used in this experiment, higher density did not inhibit the growth of *C. striata* but showed a slight promoting effect. This growth-promoting pattern can be explained by combined behavioral and physiological mechanisms and is supported by existing studies on density-dependent growth in teleosts.

Moderately elevated stocking density intensifies intraspecific feeding competition, thereby stimulating feeding motivation and increasing voluntary feed intake. This

density-driven feeding facilitation is a widely documented behavioral trait in social marine fish: group foraging competition triggers stronger appetite responses and higher overall feed consumption, thus promoting faster growth.^{29,30} A similar growth-promoting effect of increased density has also been reported for *C. striata* in recirculating aquaculture systems, consistent with the findings of this study.³¹ Second, high stocking density reduces unnecessary spontaneous locomotor activity and lowers energy expenditure for swimming and territorial behavior, thus redirecting more assimilated energy toward somatic growth. According to energy allocation theory, fish under moderate group conditions exhibit lower maintenance metabolic costs, allowing a larger proportion of ingested energy to be allocated to protein synthesis and tissue growth.³² Marchand's research on juvenile brook trout (*Salvelinus fontinalis*) thoroughly explored the effects of fish population density on energy allocation patterns.³³ The study indicated that changes in density directly alter the proportion of energy fish allocate to maintenance, activity and growth. At optimal density, the proportion of energy expended on basic vital functions and spontaneous swimming decreases, thereby increasing the share of assimilated energy available for growth. Mild density stress triggers adaptive metabolic regulation via the hypothalamic-pituitary-interrenal (HPI) axis. Moderate cortisol elevation promotes hepatic gluconeogenesis and nutrient transport, enhancing feed utilization efficiency without inducing immunosuppression.³⁴ This adaptive stress response has been widely observed in grouper and other marine carnivorous fish under moderate density conditions, where physiological indicators remain within normal ranges while growth efficiency is improved.^{35,36} It should be noted that this growth-promoting effect only exists within a suitable density range. The stable water quality regulation capacity of the recirculating system effectively buffers the accumulation of metabolic waste at high density, an important prerequisite for maintaining the positive density-growth relationship.³⁷ Beyond the threshold density, excessive crowding will induce chronic stress, suppress immune function, and ultimately inhibit growth.³⁸⁻⁴⁰ The maximum density of 50 kg m⁻³ tested in this study remains within the suitable range for grow-out *C. striata* in recirculating aquaculture.

This experiment, through orthogonal design, initially clarified the main effects of breeding size, breeding density, and feeding frequency on the survival rate and growth performance of American black sea bass. It provided a certain basis for the factory-based breeding of American black sea bass, but still had certain limitations. First, the L₉(3³) orthogonal design employed herein only allows for the assessment of main effects of stocking size, stocking density, and feeding frequency, and does not enable detection of potential interactive effects among the three factors. In commercial aquaculture practice, synergistic or antagonistic interactions among multiple operational parameters are common. Accordingly, the optimal parameter combination identified in this study reflects only main-effect trends; full factorial experimental designs are recommended in future work to disentangle factor interactions and refine parame-

ter regimes for diverse production scenarios. Second, only survival rate and specific growth rate were included as core evaluation metrics in the current study, whereas feed conversion efficiency, actual feed consumption, and physiological endpoints such as stress biomarkers and digestive enzyme activities were not systematically quantified. The inferences that elevated stocking density enhances growth via intensified intraspecific feeding competition and that feeding frequency exerts a marginal effect on survival were derived indirectly from growth performance data. These proposed mechanisms, therefore, remain speculative and require further validation through direct physiological and feeding-behavior measurements. Third, the trial was conducted in a stably operating recirculating aquaculture system with robust water-quality regulation capacity. These findings may not be directly extrapolated to other culture modes with weaker water quality buffering capacity, such as pond and cage culture. Furthermore, given the 8-week experimental duration and the 200–600 g body weight range of test fish, the conclusions are only generalizable to the medium-term grow-out phase within this size bracket; parameter optimization for larviculture and long-term on-growing of adult fish requires dedicated investigation.

5. CONCLUSION

In this study, the effects of stocking size, stocking density, and feeding frequency on the survival rate and growth performance of *C. striata* were investigated using an orthogonal design. The three factors of stocking size, stocking density, and feeding frequency had no significant effect on the survival rate. The range analysis showed that the optimal level combination was: stocking size 400 g tail⁻¹, stocking density 30 kg m⁻³, feeding frequency 2 times day⁻¹. Stocking size and stocking density had a very significant effect on specific growth rate ($p < 0.01$), and feeding frequency had no significant effect. With the increase in stocking size, the specific growth rate gradually decreased. With the increase in stocking size, it gradually increased. The range analysis showed that the optimal level combination was stocking size 200 g tail⁻¹, stocking density 50 kg m⁻³, feeding frequency 3 times day⁻¹. This study can serve as a reference for parameter optimization in industrial breeding of *C. striata*.

ACKNOWLEDGMENTS

This study was supported by a Scientific Research Project initiated by Jiangsu Coastal Development Group Co., Ltd. (No. 24210080); The authors wish to express their sincere gratitude to the anonymous reviewers whose valuable suggestions greatly improved this paper.

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ETHICAL CONDUCT APPROVAL – IACUC

The material of this paper does not involve ethical conflicts. Authors got permission to take this sample. All experimental protocols and methods in this study were approved by Chinese Academy of Fishery Sciences East China Sea Fisheries Research Institute (NO. 2026-16).

COMPETING OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

The data that has been used is confidential.

Submitted: July 22, 2026 CDT. Accepted: August 03, 2026 CDT.

Published: September 16, 2026 CDT.



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