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Original Article

The impact of stocking density on the growth performance and survival rates of hybrid grouper juveniles (♀*Epinephelus fuscoguttatus* × ♂*Epinephelus lanceolatus*) reared in composite tanks

Doan Xuan Diep[1], Le Thi Phuong Mai[2], Huynh Kim Huong[*3]

[1]Faculty of Applied Science and Technology, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.
[2]College of Rural Development, Can Tho University, Can Tho City, Vietnam.
[3]School of Agriculture and Aquaculture, Tra Vinh University, Vinh Long Province, Vietnam.

Abstract: Optimal stocking density is evaluated as a basic factor for the success of farming hybrid groupers (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂), especially in Vietnam, where this species contributes significantly to marine aquaculture. This study was performed from July to November 2024 to evaluate the impact of different stocking densities (individuals m^{-3}/ind m^{-3}), including low (6 ind m^{-3}), medium (9 ind m^{-3}), and high (12 ind m^{-3}), on the culture performance of the hybrid grouper juveniles raised in composite tanks. The juveniles, with an initial size of 4.15 g and 5.82 cm, were investigated in a 60-day completely randomized experiment with three replications. They were fed a combined diet of *Artemia* nauplii (instar I) and commercial pellet feed during the first 10 days, followed by pellet feed only for the remainder of the trial. The results showed no significant differences in survival rate (SR), feed conversion ratio (FCR), or coefficient of variation in weight (CV_W) among the different density groups ($P \geq 0.05$). Nevertheless, key growth performance indicators, including mean weight (MW), mean length (ML), daily weight gain (DWG), daily length gain (DLG), specific growth rate in weight (SGR_W), and specific growth rate in length (SGR_L), were significantly reduced at a high stocking density ($P \leq 0.05$). Additionally, there were no significant variations in rearing performance parameters between low and medium stocking densities throughout the rearing period, and a high SR range of 97.22-100% was recorded at the experimental end. The findings reliably demonstrate that hybrid grouper juveniles can be successfully reared in tank systems, with an optimal stocking density of up to 9 ind m^{-3}.

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Introduction

The hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂), also known as the Hulong grouper in Malaysia (Ch'ng and Senoo, 2008) and the Tran Chau grouper in Vietnam (Dennis et al., 2020), was developed in Malaysia in 2007. Since then, this fish has become an important aquaculture species and has been widely farmed across Southeast and East Asia, including Vietnam (Sadovy, 2013; Bunlipatanon and U-taynapun, 2017; Dennis et al., 2020; Thai et al., 2021; Zhang et al., 2024). Its popularity in aquaculture is largely due to its exceptional hybrid vigor, which is characterized by rapid growth rates (De et al., 2014; Bunlipatanon and U-taynapun, 2017; Zhang et al., 2023), improved disease resistance (Harikrishnan et al., 2010; Kim et al., 2020), tolerance to low salinity and pH conditions (Liang et al., 2013; Mustafa et al., 2013), and adaptability to various feed types and farming systems (Fitriyani et al., 2015). Furthermore, due to its outstanding quality flesh, it became a favored fish species with high market prices (Sadovy 2013).

The hybrid grouper was introduced in Vietnam as an alternative to high-risk shrimp farming (Tuan, 2004), and it has since become a well-known species in the marine aquaculture field, with production exceeding 7,000 tons and a market value estimated at US$70-105 million in 2017 (Dennis et al., 2020; Thai et al., 2021). This rapid expansion has been driven by advances in breeding techniques (Hasan, 2012;

[*]Correspondence: Huynh Kim Huong
E-mail: hkhuong77@tvu.edu.vn
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Rimmer and Glamuzina, 2019; Dennis et al., 2020). They are often intensively cultured in sea cages or earthen ponds, typically fed trash fish, with survival rates reaching up to 70% (Dennis et al., 2020). Despite its promise, the farming hybrid grouper faces numerous challenges, including a scarcity of fingerlings due to difficulties in larval rearing and increased feed costs resulting from reliance on imported industrial feed (Dennis et al., 2020). Intensive systems in ponds and cages confront continual issues in balancing high productivity and environmental sustainability (Yue and Shen, 2022; Chen et al., 2022; Cherian et al., 2023). Additionally, farming systems are under increasing pressure due to limited resources such as land, water, and feed, as well as changing weather patterns caused by climate change (IPCC, 2007; Adhikari et al., 2018).

Previous research on various grouper species has emphasized that appropriate stocking density is influenced by multiple factors, including species, developmental stage, and farming methods, making it difficult to establish a universal standard (Samad et al., 2014; Salari et al., 2012; Shao et al., 2019; Imlani et al., 2022). Particularly, data on tank-based culture systems for the groupers remain limited. It is also widely recognized that tank culture systems help reduce environmental risks by limiting polluted runoff. They also offer flexible and stable production conditions, even in climate-sensitive areas (McLean, 2021). These benefits make tank-based systems a promising option for promoting sustainable aquaculture in the face of increasing environmental and resource challenges. Therefore, this study examined the effect of varying stocking densities on the rearing performance of Tran Chau grouper juveniles in a composite tank system, aiming to gather critical data to inform the establishment and optimization of this hybrid grouper aquaculture in Vietnam.

Materials and Methods

Experimental materials: Tran Chau hybrid grouper juveniles, with an average size of 4.15±0.21 g and 5.82±0.25 cm, were obtained from a commercial hatchery in Ninh Thuan Province, Central Vietnam, where they had been maintained at a salinity of 30‰. The juveniles were transported to the experimental hatchery of Tra Vinh University in sealed, oxygen-filled plastic bags. Each bag contained 40-45 liters of water at a salinity of 30‰ and approximately 3 kg of fish. The juveniles were then gradually acclimated to a target salinity of 15‰ by lowering it by 2‰ daily. The 15‰ salinity water used for rearing was prepared by mixing seawater with freshwater. The seawater sourced from river water was filtered to remove sediment, disinfected with potassium permanganate ($KMnO_4$) at a concentration of 5 mg L^{-1}, and continuously aerated for three days. The freshwater used was city tap water. The mixed water was supplied to the rearing tanks across a cotton filter layer. Starfeed commercial pellet feed (containing 45% crude protein and produced by the Vietnam CP company) and instar I-Artemia nauplii (hatched at the experimental hatchery) were used as feeds for the trial fish. Additionally, round composite tanks with a capacity of 1 m^3 were utilized as rearing tanks, each maintained at a water depth of 0.8 m and continuously aerated.

Experimental design: This study was performed from July to November 2024 at the Experimental Hatchery of the Aquaculture Research Centre, Tra Vinh University, southern Vietnam. The 60-day completely randomized experiment with three replicates was designed to assess the effects of various densities on the growth and survival rates of hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂) juveniles reared in composite tanks. The details of the experiment are described as follows: Eighty-one health-acclimatized hybrid grouper juveniles were randomly distributed across nine rearing tanks at three different stocking densities of 6, 9, and 12 ind m^{-3}. The fish were hand-fed to apparent satiation twice daily at 7:30 AM and 4:00 PM. During the first 10 days, their diet consisted of a mix of Starfeed pellet feed and natural feed (*Artemia* nauplii, instar 1, provided at a feeding density of 6-7 nauplii mL^{-1} of water). For the

Table 1. Water quality parameters during rearing periods at various stocking densities.

Parameters	Test Time	Densities (ind m^{-3}) 6	9	12
Temperature (°C)	7:00	29.52±0.50	29.53±0.50	29.54±0.51
	14:00	30.39±0.65	30.55±0.52	30.54±0.50
pH	7:00	8.34±0.12	8.35±0.11	8.35±0.12
	14:00	8.45±0.16	8.52±0.10	8.47±0.14
TAN (mg L^{-1})		0.02±0.01	0.02±0.01	0.03±0.01
NO_2^- (mg L^{-1})		0.01±0.00	0.02±0.0.00	0.03±0.01

Values are presented as mean±SD.

remainder of the experimental time, they were fed pellets only. Feeding rate was calculated at approximately 5-7% of their body weight. Leftover feed and waste materials were removed from the rearing tanks after 2 hours of feeding. Additionally, 50% of the water in the rearing tanks was replaced twice a day during the trial period.

Collecting data: During the trial, important water quality parameters were continuously observed. Temperature and pH were measured twice daily, at 7:00 and 14:00, using a digital pH meter. Every three days at 7:00, TAN and NO_2^- levels were measured using a Sera test kit from Germany.

Five fish from each tank were randomly sampled every 15 days to test growth performance. Individual weight was measured using a digital scale with a precision of 0.01 g, and overall length was measured to the nearest millimeter with a graduated ruler. At the end of the experiment, data on the survival rate, feed conversion ratio, productivity, and coefficient of variation in weight were documented. The parameters observed were calculated using the following formulas (Stickney, 2000; Hieu et al., 2022):

Mean weight (MW, g) = total weight of 30 fish / 30

Mean length (ML, cm) = total length of 30 fish / 30

Weight gain (WG, g) = final weight - initial weight

Length gain (LG, cm) = final length - initial length

Daily weight gain (DWG, g day^{-1}) = (final weight - initial weight) / number of rearing days

Daily length gain (DLG, cm day^{-1}) = (final length - initial length) / number of rearing days

Specific growth rate in weight (SGR_W, % day^{-1}) = ((ln(final weight) - ln(initial weight)) / number of rearing days) × 100

Specific growth rate in length (SGR_L, % day^{-1}) = ((ln(final length) - ln(initial length)) / number of rearing days) × 100

Survival rate (SR, %) = (final fish number / initial fish number) × 100

Feed conversion ratio (FCR) = total dry feed given (g) / total wet weight gain of fish (g)

The coefficient of variation in weight (CVw, %) = (standard deviation /mean body weight) × 100

The animal experiments were conducted in accordance with relevant national and international guidelines. Only the Tran Chau hybrid grouper juveniles underwent weighing and measuring during the experiment, ensuring no harm was caused. After the experiments, the fish were returned to the storage tanks for further investigation.

Analyzing data: All variables were analyzed using one-way ANOVA with a significance level of $P\leq 0.05$. Duncan's multiple range test was applied to identify significant differences among stocking density means. Levene's test was used to verify the homogeneity of variances, and percentage data were arcsine-transformed prior to analysis. All statistical analyses were performed using SPSS version 20.0 for Windows.

Results

was determined by comparing the growth increment readings on scales by two readers. It was observed that the number of annuli

Water quality parameters: Throughout the trial, water temperature was maintained between 29.52 and 30.55°C, with pH levels ranging from 8.34 to 8.52. Total ammonia-nitrogen (TAN) concentrations

Table 2. Mean weight (MW) of Tran Chau hybrid grouper juveniles during the 60-day rearing at various stocking densities.

Densities (ind m^{-3})	6	9	12
MW15 (g ind^{-1})	12.33 ± 2.64^{b}	14.13 ± 2.77^{b}	9.60 ± 3.04^{a}
MW30 (g ind^{-1})	20.33 ± 3.67^{b}	22.67 ± 5.49^{b}	15.53 ± 4.68^{a}
MW45 (g ind^{-1})	25.53 ± 4.81^{b}	26.67 ± 5.78^{b}	17.53 ± 5.18^{a}
MW60 (g ind^{-1})	30.73 ± 3.45^{b}	31.33 ± 5.60^{b}	27.62 ± 7.27^{a}

Values are presented as mean±SD. Values with different letters (a, and b) in the same row show a significant difference ($P\leq0.05$).

Table 3. Mean length (ML) of Tran Chau hybrid grouper juveniles during the 60-day rearing at various stocking densities.

Densities (ind m^{-3})	6	9	12
ML15 (cm ind^{-1})	8.87 ± 0.91^{b}	8.53 ± 0.91^{b}	7.20 ± 1.14^{a}
ML30 (cm ind^{-1})	9.87 ± 0.33^{b}	9.93 ± 0.79^{b}	9.00 ± 1.36^{a}
ML45 (cm ind^{-1})	11.20 ± 0.77^{b}	$11.48\pm1.06_{b}$	9.47 ± 0.9^{a}
ML60 (cm ind^{-1})	12.33 ± 0.97^{b}	12.40 ± 0.63^{b}	10.71 ± 1.29^{a}

Values are presented as mean±SD. Values with different letters (a, and b) in the same row show a significant difference ($P\leq0.05$).

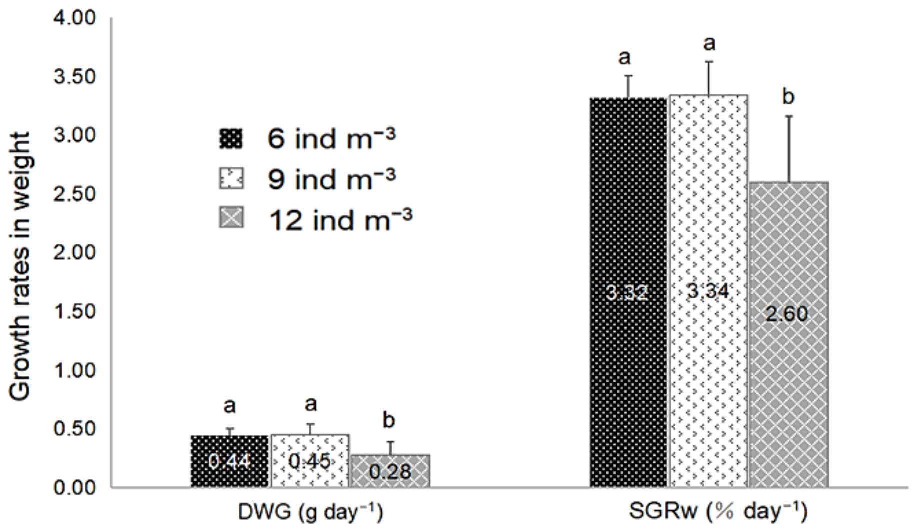

Figure 1. Daily weight gain (DWG) and specific growth rate in weight (SGRw) of Tran Chau hybrid grouper juveniles after the 60-day rearing at various stocking densities. The bars with different letters (a, b) in the same group show a significant difference ($P\leq0.05$).

remained between 0.02 and 0.03 mg L^{-1}, while nitrate-nitrogen (NO_2^-) levels ranged from 0.01 to 0.03 mg L^{-1} (Table 1).

Rearing performance: Tables 2 and 3 show that MW and ML were significantly reduced at the high stocking density compared to the low and medium densities after just 15 days of rearing ($P\leq0.05$). However, no significant differences in MW and ML were observed between the low and medium stocking densities throughout the experiment ($P\geq0.05$).

Growth rate followed a similar pattern. All growth parameters, including DWG, SGR_W, DLG, and SGR_L at the high stocking density, were significantly lower than those at the low and medium stocking densities. Nevertheless, no significant differences were found between the low and medium stocking densities for any of these parameters by the end of the experiment ($P\leq0.05$, Figs. 1 and 2).

After 60 days of rearing, FCR and CV_W showed no significant differences among the three stocking densities ($P\geq0.05$). Additionally, no mortality occurred at the low and medium stocking densities. While a slightly lower mortality rate was observed at the high stocking density, the difference was not significant ($P\geq0.05$) (Table 4).

Discussions

In aquaculture, determining the ideal stocking density for each species in specific farming systems (e.g., ponds, tanks, cages) is economically vital, as it

Table 4. Feed conversion ratio (FCR), coefficient of variation in weight (CVW) and survival rate (SR) of Tran Chau hybrid grouper juveniles after the 60-day rearing at various stocking densities.

Densities (ind m^{-3})	**6**	**9**	**12**
FCR	0.99±0.01^{a}	1.00±0.03^{a}	1.05±0.05^{a}
CVw	12.00±1.0^{a}	19.00±1.73^{a}	30.33±17.39^{a}
SR (%)	100±0.00^{a}	100±0.00^{a}	97.22±4.61^{a}

Values are presented as mean±SD. Values with different letters (a, b, c) in the same row show a significant difference (P≤0.05).

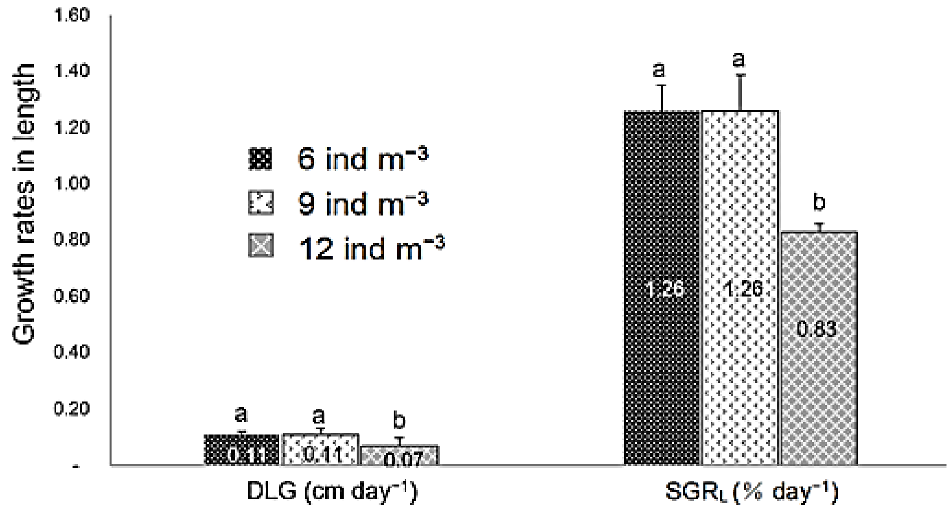

Figure 2. Daily length gain (DLG) and specific growth rate in length (SGRL) of Tran Chau hybrid grouper juveniles after the 60-day rearing at various stocking densities. Values are presented as mean±SD. The bars with different letters (a, b) in the same group show a significant difference (*P*≤0.05).

enables cost optimization while ensuring fish welfare (Biswas et al., 2015; Yang et al., 2020; Paul et al., 2021; Hossain et al., 2022). Lower stocking densities can reduce social interactions, competition for feed and space, and negative impacts on water quality. However, they frequently necessitate bigger facility investments per unit of product. They may limit overall biomass yield due to a smaller initial fish stocking amount, potentially resulting in increased production costs. Additionally, extremely low densities can disrupt social structures and increase aggressive behavior among fish (Chakraborty et al., 2010; Aragón-Flores et al., 2014). Conversely, high stocking densities are commonly used to maximize land and water resource utilization. However, high stocking densities can negatively affect the performance and welfare of farmed fish by inducing stress and altering physiological responses, which in turn can impair health and reduce growth and survival rates (Liu et al., 2019; Yang et al., 2020; Montenegro et al., 2022; Ly et al., 2024).

In the present study, we evaluated the culture performance of Tran Chau hybrid groupers reared at low, medium, and high stocking densities (6, 9, and 12 ind m^{-3}, respectively) in composite tanks. A high SR ranging from 97.22 to 100% was recorded in the tested stocking density range. However, all growth performance parameters were significantly reduced at the high stocking density. Fish health indicators are frequently expressed via physiological responses, which are directly linked to growth performance (Jia et al., 2022). Through numerous physiological routes, high stocking density is a chronic stressor and has led to an inhibition in growth rates in raised aquatic animals (Jia et al., 2016; Ezhilmathi et al., 2022; Jia et al., 2022; Diao et al., 2023). For example, high-density conditions induced physiological responses, oxidative stress, and irregular hepatic lipid metabolism in *Micropterus salmoides* fingerlings (Jia et al., 2022) and *Pelteobagrus fulvidraco* (Diao et al., 2023) reared in an integrated rice–fish farming system over a 90-day period. Similarly, high stocking density over 80 days in a land-based recirculating aquaculture system led to reduced growth performance, elevated plasma biomarkers, impaired liver function, and increased oxidative stress in juvenile turbot (*Scophthalmus maximus*) (Jia et al., 2016). Additionally, fish reared in higher stocking densities displayed lower digestive enzyme activities (protease, amylase, lipase, and cellulase) in Asian seabass (*Lates calcarifer*), as reported by Ezhilmathi et al. (2022).

FCR is a vital indicator used to evaluate efficiency in aquaculture. It reflects how much feed is required to produce a certain amount of biomass from farmed aquatic species. A lower FCR indicates improved culture efficiency, as it is associated with reduced feed input, minimized waste output, supported better water quality, and helped lower both operational costs and environmental burden (Besson et al., 2016; de Verdal et al., 2017). In the present study, FCR showed no significant differences within a stocking density range of 6 to 12 ind m^{-3}. Remarkably, the FCR values ranged from 0.00 to 1.00, showing an improvement over the 1.21-2.10 range reported by Noor et al. (2018), who raised the hybrid grouper in tanks with different salinities and feeds at a higher stocking density (15 fish/356 L tank) than that of our settings. This FCR improvement may help explain the low and stable concentrations of total ammonia nitrogen (TAN) (0.02-0.03 mg L^{-1}) and nitrite nitrogen (NO_2^-) (0.01-0.03 mg L^{-1}) observed during this trial.

On the other hand, CV, a growth performance, is commonly used to assess size uniformity among individuals in farmed fish populations. A low CV indicates relatively uniform body sizes, which is beneficial for feeding efficiency, growth performance, and synchronized harvesting. In contrast, a high CV signifies greater size variation, which can reduce production efficiency and negatively affect market value (Gomez and Gomez, 1984; Santos and Dias, 2021). In the present study, the CV for body weight (CV_W) ranged from 12.00 to 30.33%, which was within the common range reported for many fish species. According to Gjedrem (1997), CV values in farmed livestock are generally much lower, around 7-10%, whereas in most fish species, the CV is considerably higher, at 20-35%.

Groupers are known for their territorial behavior and are considered fierce predators in crowded conditions (Paruntu et al., 2018; Darwin and Padmavathi, 2020). However, the present study demonstrated that the tested range of stocking densities had no significant effect on the survival rate (SR) of Tran Chau hybrid grouper juveniles. The observed SR of 97.22-100% aligns with previous findings by Othman et al. (2015) and Ismail et al. (2018). In addition, the SGR recorded in this study, ranging from 3.32 to 3.34% day^{-1}, is slightly higher than the values 2.83-3.24% day^{-1} reported by Ebi et al. (2018) and markedly higher than those (0.40-1.05% day^{-1}) reported by Noor et al. (2018) and 1.95-2.80% day^{-1} (Othman et al., 2015).

Key water quality parameters were maintained within optimal ranges for Tran Chau grouper throughout the trial period, including a temperature of 29.52-30.55°C, pH of 8.34-8.52, TAN at 0.02-0.03 mg L^{-1}, and nitrite-nitrogen (NO_2^-) at 0.01-0.03 mg L^{-1} (Ismail et al., 2018; Noor et al., 2018; Zhang et al., 2018; Das et al., 2021). These findings indicate that rearing Tran Chau hybrid grouper juveniles in composite tanks is highly feasible at optimal stocking densities of up to 9 ind m^{-3}.

Conclusions

A high stocking density (12 ind m^{-3}) significantly reduced the growth performance of the trial fish, whereas no significant differences were observed in rearing performance parameters between the low (6 ind m^{-3}) and medium (9 ind m^{-3}) stocking densities. These findings suggest that rearing Tran Chau hybrid grouper juveniles in tank systems is highly feasible at stocking densities of up to 9 ind m^{-3}. Overall, the study provides foundational data on appropriate stocking densities to support the development and optimization of hybrid grouper aquaculture in tank-based systems. Further studies on biofloc or hydroponic tank systems are recommended to improve this fish's rearing performance.

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