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Effect of Stocking Density on Growth, Feed Efficiency, and Survival in Peruvian Grunt *Anisotremus scapularis* (Tschudi, 1846): From Fingerlings to Juvenile

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Abstract: This study aimed to identify the effect of stocking density on growth, feed efficiency, and survival during the transition from Peruvian grunts (*Anisotremus scapularis*) fingerlings to juveniles. Fingerlings were reared in triplicate for 63 days until reaching the juvenile stage, at 1000 fingerling·m^{-3} (low-density LSD, 0.79 kg·m^{-3}), 2000 fingerlings·m^{-3} (medium-density MSD, 1.58 kg·m^{-3}), and 3000 fingerlings·m^{-3} (high-density HSD, 2.37 kg·m^{-3}), and production performance parameters were evaluated. At the end of the experiment, results showed a negative correlation between stocking density and growth, individual growth, and the specific growth rate for HSD. The final biomass per treatment was 3.53 ± 0.26, 6.79 ± 0.08, and 7.70 ± 0.46 kg·m^{-3} for LSD, MSD, and HSD, respectively, the biomass harvest and weight gain were significantly lower for HSD. At the end of the experiment, there was no significant difference in survival (99%) among all treatments. Furthermore, the average food for each individual and the protein efficiency ratio were significantly lower for HSD, while the feeding efficiency was higher for HSD. In summary, our results indicated that initial biomass values above 1.42 kg·m^{-3} did not significantly improve growth and feed efficiency in the fingerlings rearing process.

Keywords: peruvian grunt; stocking density; fingerlings; juvenile; growth; feed efficiency

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1. Introduction

Aquaculture has been established as a feasible strategy to supply seafood to the consumer as a sustainable alternative to fisheries [1]. In addition, an expansion in the diversification of locally grown species increased in recent years, reaching 415 farmed species in 2017 [2]. Thus, species of the family Haemulidae, such as the eastern sweetlips (*Plectorhinchus vittatus*) in the Indian Ocean [3], the spotted grunt (*Pomadasys commersonnii*) in the southeast coast of South Africa [4], the French grunt (*Haemulon flavolineatum*) porkfish (*Anisotremus virginicus*) in the western tropical North Atlantic Ocean [5], or the Peruvian grunt (*Anisotremus scapularis*) in the southeast Pacific Ocean [6] were considered for its inclusion in aquaculture due to their local economic importance.

The genus Anisotremus Gill 1861 contains ten species [7,8], characterized by inhabiting predominantly subtropical coral and rocky reefs [8]. The Peruvian grunt (*A. scapularis*) is a bento-pelagic species from coastal areas and rocky bottoms. Its distribution ranges from Ecuador, including Cocos Islands, the Galapagos, to Antofagasta in Chile [9]. It is a euriphagic species, preferably on gastropods, polyplacophorans, crustaceans, echinoderms, fish, and algae, considered a general carnivore [10]. The *A. scapularis* is one of the five species of Anisotremus in Peru, so to initiate domestication processes of the species, it is advisable to know its phylogenetic relationships [8] as well as the unequivocal identification of the species through the analysis of mitochondrial genes [11].

Likewise, the Peruvian grunt (*A. scapularis*) is a species highly appreciated by consumers in Peruvian and Chilean gastronomy [12]. In recent years, a substantial increase in landings of this species has been observed, from 87 metric tons in 2010 to 498 in 2019 [13]. In addition, breeders show optimal captivity acclimatization, handling, a high number and frequency of female spawning, and high egg fecundity in tanks [6,14], thus obtaining adequate criteria to be considered a candidate species for its domestication [15]. However, to farm new species, it is necessary to establish efficient protocols and implement improvements in each of the domestication phases. Maximizing rearing animal growth [16], optimizing feed consumption [17], and identifying the appropriate stocking density are critical issues for the new species to be considered profitable for companies.

Stocking density is one of the main impact factors, related to growth and feeding efficiency, on achieving the time required for attaining targeted market sizes [18]. This is a critical issue in the industrial production of new species profitability since reaching the sale size and the expense associated with feeding determines the success of a farmed species [19]. Therefore, the effects of stocking densities on growth for those species currently established in the sector, such as the rainbow trout (*Oncorhynchus mykiss*) [20], Nile tilapia (*Oreochromis niloticus*) [21], or the Atlantic salmon (*Salmo salar*) [22], were rigorously analyzed. In addition to promising species such as cod (*Gadus morhua*) [23], halibut (*Hippoglossus hippoglossus* L.) [24], juvenile red sea bream (*Pagrus major*) [25], gilthead seabream (*Sparus aurata*) [26,27], and crucian carp, (*Carassius carassius*) [28], the confirmation of decreased growth associated with the initial stocking density was a limiting factor for their industrial farming.

Thus, food constitutes more than half of the operational costs of farmed species such as Nile tilapia [29], Atlantic salmon [30], or sea bream [31], and feeding efficiency is key to the viability of farming at new species. Both are considered indicators of the estimation between the conversion of the feed dispensed into the biomass harvest production [32]. Since the Peruvian grunt is a potential species for aquaculture, there is a lack of information on the effect of these key parameters to analyze its farming viability.

Therefore, this study aimed to evaluate the effect of the stocking density on growth, feeding efficiency, and survival during the rearing of Peruvian grunt fingerlings.

2. Materials and Methods

2.1. Animals and Husbandry

The broodstock comprised 11 breeders (7 females and 4 males) caught by artisanal fishermen, and it has been acclimatized since 2016 in the aquaculture facilities of the Morro Sama-FONDEPES Aquaculture Center (−17.99° S; −70.88° W; Morro Sama-Tacna, Peru). The breeders were kept in a 6000 L cylindrical tank in a continuous flow-through seawater system at a temperature of 18 ± 2 °C. The tank was covered with raschel mesh, reaching 330–350 lux light intensity. Feeding of the breeders began with life prey Pacific sand crab (*Emerita analoga*) combined with manufactured food (see Section 2.4. Feeding), progressively removing the live prey until they were only fed the inert diet. After six months, the first spontaneous spawning was obtained [6]. Then, 10,000 larvae, hatched of the same eggs batched from an undifferentiated female/females, were reared in a 1000 L cylindrical tank at 10 larvae·L^{-1} in continuous flow-through seawater, central soft aeration, artificial light conditions with an intensity of 600 lux, and a photoperiod of 18:6 (Light:Dark). Larvae were initially fed with rotifer (*Brachionus* sp.), and at 18 DPH (days post hatching), with nauplii of brine shrimp (*Artemia* sp.). Larvae at 31 DPH began weaning using micropellets manufactured according to Section 2.4. Then, 5400 Peruvian grunt fingerlings were selected at 90 DPH to perform the stocking density trial.

2.2. Experimental Designed

The experimental plan was balanced monofactorially, with three replicates per treatment level using nine cylindrical tanks of 500 L each, at a final seawater volume of 300 L. The fingerlings were kept in continuous flow-through seawater, with total renewal every hour at a temperature of 16 ± 1 °C and dissolved oxygen in the range of 6 ± 1 mg·L^{-1}. The fish were kept in ambient light conditions with an intensity of 500 ± 200 lux. The stocking densities for each treatment were 1000 fingerlings·m^{-3} (low density, LSD, 0.79 kg·m^{-3}), 2000 fingerlings·m^{-3} (medium density, MSD, 1.58 kg·m^{-3}), and 3000 fingerlings·m^{-3} (high density, HSD density, 2.37 kg·m^{-3}). Therefore, individuals were randomized into three tanks per treatment, N = 900 for LSD, N = 1800 for MSD, and N = 2700 for HSD. In addition, a sample of fingerlings (N = 225) from each treatment was anesthetized (see below), weighed (wet weight), and measured (total length) using 2-phenoxyethanol (0.3 mL·L^{-1}; Sigma®, St. Louis, MO, USA), a precision scale (Mettler UMT2 ± 0.1 mg, Toledo, Switzerland), and an ichthyometer (KH-PISCIS-RIO (M); Krauss & Henke; range 0–40 cm, precision 1 mm), respectively. Then the trial period ranged from 13 July to 13 September 2017 (9 weeks). At 30 days from the beginning of the experiment and at the end of this period, measurements of the total length (cm) and wet weight (g) were obtained, and mortality was verified daily. The fingerlings were anesthetized in a solution of 2-phenoxyethanol independently for each treatment to guarantee traceability. Afterward, fingerlings were kept in a 50 L tank until they remained actively swimming before being transferred to their initial tank.

2.3. Growth and Survival

For growth analysis at different stocking densities, the following parameters were evaluated:

Weight gain (WG%)

$$WG\% = ((MBWWf\text{-}MBWWi)/MWBWi) \times 100 \quad (1)$$

where MWBWf is mean of the final body wet weight and MWBWi is the mean of the initial body wet weight (%).

Individual Growth (IG)

$$IG = BWWf - BWWi \quad (2)$$

where BWWf is the final body wet weight and BWWi is the initial body wet weight (in g).

Specific growth rate (SGR%)

$$SGR\% = ((LnBWWf - LnBWWi)/t) \times 100 \quad (3)$$

where t is the duration of the experiment in days, BWWf is the final average individual body wet weight, and BWWi is the initial average individual body wet weight (in % BWW day^{-1}).

Biomass harvest (%)

$$Bh\% = ((Bf - Bi)/Bf) \times 100 \quad (4)$$

where Bh% is the mean of biomass harvest, Bf is the final biomass, and Bi is the initial biomass (g).

Survival (%)

$$S(\%) = (Nf/Ni) \times 100 \quad (5)$$

where Nf is the number of Peruvian grunt fingerlings at the end of the experiment and Ni is the initial number.

2.4. Feeding

The feed ingredients (Table 1) were homogenized in a crusher (tk32 plus, Henkel, Henkel Peruana S.A., Lima, Peru), making 0.2-cm-wide strips. The proximal feed pellets' composition is shown in Table 1. Afterward, the pellet strips were dried at 30 ± 10 °C until reaching an average humidity of around 10%. Later, the pellets were crushed and classified using a sieve (IRIS FTL-0200, Santiago, Chile) until sizes between 1 and 2 mm were obtained. At the beginning of each day, 100 g of pellets were weighed to feed the fish in each tank. Fingerlings were fed manually three times a day (8:00 a.m., 2:00, and 8:00 p.m.) 7 days a week until satiety. At the end of the day, the remaining amount of food for each tank was weighed in order to obtain the total food weight consumed daily. The tanks were siphoned at the beginning of each day (from 7:00 to 8:00 a.m.) to eliminate uneaten pellet residues and remove faces, in order to maintain optimal seawater conditions.

The feeding parameters were evaluated using the following formulae:

Feeding efficiency (FE%)

$$FE\% = (BG/FU) \times 100 \quad (6)$$

where BG is the biomass gained (g) and FU is the feed consumed by fingerlings (g).

Feed conversion ratio (FCR)

$$FCR = TFG/TWG \quad (7)$$

where TFG is the total feed consumed and TWWG is the total wet weight gained.

Protein efficiency ratio (PER)

$$PER = WG/PF \quad (8)$$

where WG is the weight gained by the fingerlings (g) and PF is the protein feed consumed by fingerlings (g).

Table 1. Artisan-manufactured pellets' formulation and composition used for the study.

Ingredient	Percentage (%)
Fish meal	61.98
Soybean meal	20.66
Wheat meal	1.15
Fish oil	12.78
Gelatine *	0.65
Vitamin C	0.51
Vitamin E	0.04
Vitamin B	0.44
Mineral mix	1.48
Methionine	0.10
Threonine	0.10
Lysine	0.10
Proximate analysis	
Ash	12.86
Crude lipid	14.49
Crude protein	52.29
Cellulose	1.24
Moisture	8.81

* Mammalian gelatine.

2.5. Statistical Analyses

All data series were checked for normal distribution using the one-sample Kolmogorov–Smirnoff test, as well as for homogeneity of variances using Levene's test [33]. When necessary, an *arcsin* transformation of data was performed [33]. Growth, feeding, and survival were compared with a one-way ANOVA analysis, using the statistical package STATISTICA 10.0© [33]. Pairwise means were compared with Tukey's test, and the nominal level for significance was settled at $p \leq 0.05$.

3. Results

3.1. Growth, Biomass, and Survival

The growth performance of Peruvian grunt fingerlings was significantly affected by the experimental stocking densities tested (Table 2). At the beginning of the experiment, the individual wet weight and the total length were homogeneous among all the treatments (Table 2). After one month, the average weight and total length per individual were significantly different between all densities. At the end of the experiment (63 days), both the average wet weight and total length were significantly lower only for HSD (Tukey HSD test, $p < 0.01$). Thus, the fingerlings exposed to different densities (LSD, MSD, and HSD) showed exponential growth (Figure 1), reaching a better adjustment at a lower density ($R^2 = 0.99$ for LSD, $R^2 = 0.98$ for LSD, and $R^2 = 0.96$ for HSD). The average individual growth (IG) was higher for LSD (2.74 + 0.22 g), and MSD (2.61 + 0.02 g) showed significant differences compared to HSD (Tukey HSD test, $p < 0.01$). Furthermore, the percent of the specific growth rate (SGR%) was significantly lower for HSD (Tukey HSD test, $p < 0.01$) with values of 0.024 ± 0.002, 0.023 ± 0.001, and 0.019 ± 0.001%·day^{-1} for LSD, MSD, and HSD, respectively. The final biomass per treatment was 3.53 ± 0.26, 6.79 ± 0.08, and 7.70 ± 0.46 kg·m^{-3} for LSD, MSD, and HSD, respectively. The percentages of biomass harvest and weight gain were significantly lower for HSD (both Tukey HSD test, $p < 0.01$; Table 2). At the end of the experiment, there was no significant difference in survival among all treatments for all stocking densities of 99% (Table 2).

Table 2. Growth parameters and survival of Peruvian grunt (*A. scapularis*) measured during the transition to fingerlings from the juvenile reared trial (63 days) at low stocking density (LSD), medium stocking density (MSD), and high stocking density (HSD).

Parameters	LSD	MSD	HSD	F-Value	*p*
Initial average length (cm)	4.08 + 0.14	4.15 + 0.03	4.15 + 0.03	0.62	0.57
Middle (30 days) average length (cm)	4.97 + 0.04 ^{a}	4.78 + 0.06 ^{b}	4.53 + 0.03 ^{c}	70.62	<0.001
Final average length (cm)	6.48 + 0.42 ^{a}	6.35 + 0.53 ^{a}	5.81 + 0.65 ^{b}	27.38	0.001
Initial average wet weight (g)	0.79 + 0.06	0.79 + 0.04	0.79 + 0.01	0.00	1.00
Middle (30 days) average wet weight (g)	1.50 + 0.03 ^{a}	1.34 + 0.06 ^{b}	1.12 + 0.01 ^{c}	83.7	<0.001
Final average wet weight (g)	3.53 + 0.17	3.40 + 0.03	2.57 + 0.20	35.54	<0.001
Individual Growth (g)	2.74 + 0.22 ^{a}	2.61+ 0.02 ^{a}	1.78 + 0.19 ^{b}	29.15	<0.001
Specific growth rate (%·day^{-1})	2.38 + 0.18 ^{a}	2.33 + 0.06 ^{a}	1.87 + 0.10 ^{b}	14.84	0.005
Biomass gained (g)	822.84 ± 50.96 ^{a}	1562.55 ± 18.23 ^{b}	1599.12 ± 183.51 ^{b}	47.17	<0.001
Biomass harvest (%)	77.60 ± 1.06 ^{a}	76.72 ± 0.21 ^{a}	69.10 ± 2.42 ^{b}	28.08	<0.001
Weight gain (%)	349.57 ± 51.85 ^{a}	330.15 ± 16.51 ^{a}	224.73 ± 21.69 ^{b}	11.84	0.008
Survival (%)	99 + 0.33	99 + 0.10	99 + 0.29	3	0.16

Values are means ± SD (N = 3), different letters in the same row signify statistical differences (F-value of one-way ANOVA, $p < 0.05$).

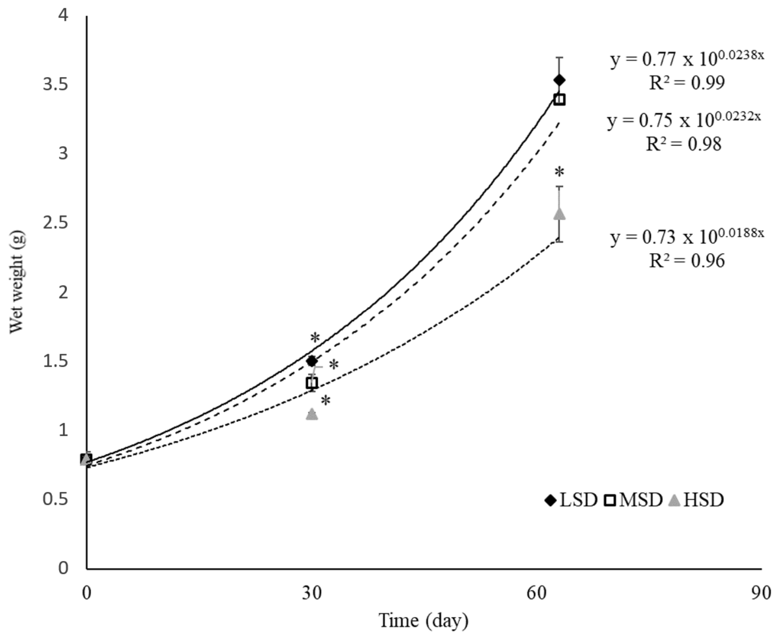

Figure 1. Growth trend in the wet weight of Peruvian grunt (*A. scapularis*) fingerlings rearing over 63 days to reach the juvenile stage at low stocking density (LSD), medium stocking density (MSD), and high stocking density (HSD). Values are means ± SD of triplicates, significant differences among growth are indicated by an asterisk * by one-way ANOVA ($p < 0.05$). LSD.

3.2. Feeding

The total food intake in each treatment was 3912.30, 7202, and 10,477.06 g for LSD, MSD, and HSD, respectively (Table 3). Thus, the average amount of food for each individual and feeding efficiency were significantly lower for HSD (Tukey HSD, $p < 0.01$) compared to LSD and MSD (Table 3). Furthermore, HSD showed a significantly lower feeding efficiency (FE%) (Tukey HSD test, $p < 0.01$) compared to the other stocking densities (Table 3). However, the protein efficiency ratio (PER) was significantly lower for HSD, being 0.88 + 0.09 compared to LSD and MSD (1.21 + 0.09 and 1.25 + 0.00, Table 3).

Table 3. Feeding parameters measured during the cultivation process (63 days) at low stocking density (LSD), medium stocking density (MSD,), and high stocking density (HSD) of Peruvian grunt (*A. scapularis*) fingerlings.

Parameters	LSD	MSD	HSD	F-Value	*p*
Total food consumed (g)	3912.3 [a]	7202 [b]	10,477.06 [c]	14,343.1	<0.001
Food average consumed per indv. (g)	4.35 ± 0.02 [a]	4 ± 0.01 [a]	3.88 ± 0.03 [b]	349.7	<0.001
Feeding efficiency (%)	63.09 + 4.68 [a]	65.15 + 0.21 [a]	49.61 + 5.17 [b]	13.17	0.006
Feed conversion ratio (FCR)	1.59 + 0.12 [a]	1.53 + 0.00 [a]	2.20 + 0.22 [b]	19.87	0.002
Protein efficient ratio (PER)	1.21 + 0.09 [a]	1.25 + 0.00 [a]	0.88 + 0.09 [b]	19.87	0.002

Values are means ± SD (N = 3), different letters in the same row signify statistical differences (F-value of one-way ANOVA, $p < 0.05$).

4. Discussion

The effect of stocking density and feed efficiency on growth and biomass production during the transition from fingerlings to juveniles is considered a profitability evaluator for new farming candidate species. Thus, in this study, the diet used during the rearing trial met the nutritional requirements of the fingerlings and juveniles, consisting of 52.29% protein and 14.49% total lipids (Table 1). Previous studies confirmed juvenile Peruvian grunts (*A. scapularis*) and spotted grunters (*Pomadasys commersonnii*, Haemulidae) fed a diet content of approximately 50% crude protein and 15% lipids reached the best growth records [4,34,35].

Moreover, the analysis of the effect of stocking density on growth, fed efficiency, and survival during the transition from fingerlings to juveniles of the Peruvian Grunt was carried out for the first time over 63 days (from 90 to 153 DPH). Thus, our results showed a marked negative effect on the growth of fingerling Peruvian grunts reared at a high stocking density (30,000 fingerlings·m^{-3}, 2.37 ± 0.05 kg·m^{-3}), but not in survival. However, similar results have been previously reported in species proximate to the order Lutjaniformes, such as the yellow Snapper *Lutjanus argentiventris* and *Lutjanus peru* (Lutjanidae), which, at densities of 0.15 and 4.4 kg·m^{-3} respectively, significantly reduced their growth compared to lower densities [36,37]. Furthermore, a significant decrease in growth alongside an increasing stocking density was observed for species already established in farmed aquaculture such as Atlantic salmon (*Salmo salar*), which, when reared at high densities (28.79 kg·m^{-3}), significantly decreased their growth compared to medium and low densities (~19.62 Kg·m^{-3} and ~9.80 kg·m^{-3}) under controlled conditions [30]. Similarly, the juvenile European sea bass (*Dicentrarchus labrax*) was reared at different stocking densities (80, 165, 325, and 650 specimens·m^3), observing a decrease in growth at high densities [38]. Moreover, white seabream (*Diplodus sargus*) performance juveniles cultured at low (1.96 kg·m^{-3}) and high (7.79 kg·m^{-3}) or juveniles of red seabream (*Pagrus major*) growing at a low density achieved more significant growth than at a high density [25,39].

Congruently, the growth during the trial time was exponential and showed a better adjustment (R^2) to the treatments with a lower stocking density (Figure 1). Previous studies also observed reared juvenile *A. scapularis* (0.16 fish·L^{-1}) adjust to exponential growth [34]. Furthermore, a similar adjustment was observed in juveniles of the Blunt snout bream (*Megalobrama amblycephala*) exposed to different densities [40]. The average individual growth (IG) and the specific growth rate (SGR%; HSD of 0.019 ± 0.001 BW·day^{-1}) showed a significant decline in Peruvian grunt fingerlings at high densities. The SGR also decreases significantly with the increase in the stocking density (0.1, 0.3, and 0.5 fish/L) in *Orthopristis chrysoptera* (Haemulidae) reared during a period of 65 days [41]. A similar trend was also observed in *L. peru* in which the SGR (% per day) decreased by 1.07 ± 0.01, 0.95 ± 0.02, and 0.91 ± 0.03 for 30, 50, and 70 (fish/m^3) at densities of 70 fish/m^3 [37]. Furthermore, a decrease in the special growth rate (SGR) was observed in the *Salmo salar* when its growth at high densities was evaluated [30,42]. Freshwater fish species, such as the Nile tilapia, showed a specific growth rate (% SGR) negatively correlated with population density [21]. Specific growth rates for Atlantic cod (*G. morhua* L.) decreased from a maximum of 1.08% at a stocking density of 2 kg·m^{-3} to 0.66% at a density of 40 kg·m^{-3} [23]. This also occurs for marine flatfish species such as turbot (*Scophthalmus maximus*), and in Dover sole (*Solea solea*) in which a similar trend was observed [43,44].

Furthermore, there were no significant differences in the biomass gained between MSD and HSD, while the percentage of biomass harvest and weight gain was significantly lower for HSD (both Tukey HSD tests, $p < 0.01$; Table 2). This implies that the balance between reared density and growth should be considered to obtain higher production. Similar results were obtained in *Labeo bata* fingerlings reared in cages in which, at a medium stocking density (75 fingerlings·m^{-3}), they reached similar biomass values to that of a higher stocking density [45]. Thus, in cod, biomass increased between 60% and 89% in fish stocked at low densities (10 kg·m^{-3}) but only 50% in those maintained at a high stocking density of 40 kg·m^{-3} [23]. On the contrary, in the yellow snapper *L. argentiventris*, the highest biomass harvest was obtained at a high stocking density (12 fish·m^{-3}) [36], and in tilapia, a correlation was observed between the increase in stocking density and biomass harvest [46]. This effect is motivated by the increase in competition for food and space, with the increase in biomass per unit volume causing higher energy consumption, increased metabolism, and decreased food intake [47,48].

Furthermore, individual fingerlings of the Peruvian grunt at HSD consumed less food than those kept at the other two densities (LSD and MSD; Table 3). Therefore, the feeding efficiency was significantly lower at a high density. This fact may be associated with stress factors as seen in juveniles of gilthead sea bream (*S. aurata*) or induced by schooling behaviors as occurs in juvenile sea bream [27,49]. The same trend was shown in tilapia fingerlings; the effect of stocking density was negatively correlated with feeding efficiency [21]. In addition, lower food consumption may occur due to hierarchical behaviors as occurs in *D. labrax* [50].

Moreover, the feed conversion ratio (FCR) was higher for HSD compared to the other densities, while the protein efficiency ratio (PER) was significantly lower for HSD, being 0.88 + 0.09 compared to LSD and MSD (1.21 + 0.09 and 1.25 + 0.00, Table 3). A lower feed conversion ratio (FCR) equates to increased production efficiency and reduces the costs associated with fish feeding [19, 32]. Therefore, similar protein retention results (0.8 to 1.8) were reported for *P. commersonnii* for different dietary protein compositions [4]. Furthermore, *S. salar* or *L. rohita* showed higher FCR and PER as the stocking density increased [44,51]. Furthermore, in juvenile seabass at densities of 80, 165, 325, and 650 specimens·m^{-3}, a lower feed conversion rate was found for fish in the highest stocking density [38].

5. Conclusions

An increase in stocking density above MSD (1.42 ± 0.01 kg·m^{-3}) for fingerlings does not significantly increase growth and biomass produced. Thus, the fish fed at MSD and LSD had more suitable food use than HSD, thus obtaining better feeding efficiency, lower FCR, and better PER. In summary, this study indicates that during the critical growth stage from fingerlings to juveniles, it is advisable to maintain a density not exceeding 1.42 kg·m^{-3}.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to not having a public directory to share raw data.

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