Impact of short-term stunting on growth and biochemical composition of snubnose pompano *Trachinotus blochii* (Lacepede 1801)

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Abstract

The impact of stunting on growth and carcass composition of snubnose pompano *Trachinotus blochii* (Lacepede, 1801) was investigated. During the stunting phase (30 days), three tanks were stocked with 60 fish each and fed at 3% of body weight (BW) for stunting. Another set of three tanks were stocked at 20 fish per tank and fed at 10% of BW, which served as normal control. The stunted fish had lower final body weight (4.46±0.18 g) than the normal fish (5.4 ± 0.23 g) with a lower specific growth rate (%) per day (SGR), lower (p<0.05) crude lipid in carcass, higher hepatosomatic index (%) (HSI) and total protein in the liver. During post-stunting phase (30 days), stunted fish were restocked at 20 fish per tank and fed at 15% of body weight. Final length and weight of post-stunted fish increased; but with a lower SGR indicating partial compensatory growth (CG). Crude carcass lipid, crude liver protein and HSI were significantly (p<0.05) reduced during the post-stunting period. The findings of the study suggest a protein sparing trend in fish, wherein lipids stored in body and liver were utilised during the stunting phase, while both body lipid and liver protein reserves were mobilised during the CG.

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Introduction

Marine finfish culture is emerging as an ideal investment in India's coastal area due to the introduction of several new candidate species. Stunted fingerling production is becoming a commercial practice for some of the economically important fishes such as carp (Abraham et al., 2010; Das et al., 2016; Suresh Babu et al., 2021) in India. This practice has been proven technically feasible in other fish species such as milk fish (Lingam et al., 2019) and Tilapia (Bhujel et al., 2007). Stunted fingerlings are presumed to be robust and healthy stocking material that assures long term seed availability with easy portability (Radheysham and Saha, 2009). Stunting also shortens the culture period while increasing fish production. Nursery management is an important prerequisite for developing farming practices for any candidate species for aquaculture. Recently nursery rearing for a bunch of marine finfishes such cobia (Gopakumar *et al.*, 2010), silver pompano (Gopakumar *et al.*, 2011), Indian pompano (Megarajan *et al.*, 2023) and orange spotted grouper (Megarajan *et al.*, 2022), were standardised in India for marine and coastal aquafarming. Nursery rearing can be taken up in various types of rearing systems such as tanks, cages, hapas and recirculating aquaculture systems (Megarajan *et al.*, 2023) based on the requirements.

Compensatory growth (CG) is a phase of accelerated growth when favourable conditions are restored after a period of growth depression. CG is important to fisheries management, aquaculture and life history analysis because it can balance the effects of growth retardation depending on the duration of stunting (Lingam *et al.*, 2019; Anikuttan *et al.*, 2021) which may

be short-term for a few weeks (4 to 10 weeks) (Anikuttan *et al.*, 2021) or long-term for even more than six months (Das *et al.*, 2016; Lingam *et al.*, 2019; Suresh Babu *et al.*, 2021).

Snubnose pompano Trachinotus blochii (Lacepede, 1801), is recognised as a prospective species for mariculture due to its ease of adaptation to the culture environment. It readily takes artificial feeds and grows rapidly and consistently compared to other farmed fishes. Given the strong demand for premium finfish in both the local and international markets, the potential market for this high-value finfish is considerable. A variety of farming systems. including ponds (Javakumar et al., 2014; Damodaran et al., 2019), marine cages (Kalidas et al., 2020) and low saline cages (Suresh Babu et al., 2022) and recirculating aguaculture systems have been standardised for effectively raising snubnose pompano T. blochii. It is considered as an excellent candidate species for farming in both marine and low-saline conditions (Kalidas et al., 2012). Due to its euryhaline nature, high meat guality, superior processing yield, strong market demand, accessibility to commercial feed and consistent seed production, this species is considered ideal for aquaculture.

Production of stunted fingerlings of snubnose pompano has been recently reported under indoor marine conditions (Anikuttan *et al.*, 2021), indoor low-saline conditions (Suresh Babu *et al.*, 2022) and in low saline cages (Suresh Babu *et al.*, 2022). Stunted growth in cultured fish can be induced by a combination of feed restriction and limited living space. Lingam *et al.* (2019) in milkfish and Suresh Babu *et al.* (2021) in rohu demonstrated that stunting can be effectively achieved by increasing stocking density along with sub-optimal feeding. In the present study, a similar approach, combining feed restriction and higher stocking density was adopted for stunted fish production.

The current study investigates the impact of short-term stunting of snubnose pompano in indoor tanks on water quality, growth performance and carcass composition. The study aims to provide new insights on the management of stunted fish, that has not been previously explored in detail.

Materials and methods

Experimental protocol

The experiment was conducted in indoor conditions in two phases, such as stunting phase and the post-stunting phase. Fibre reinforced plastic (FRP) tanks of 300 I capacity, each filled with 200 I of clean filtered and chlorinated low saline water (15 ppt) were used for the experiments. The water was chlorinated with 10 ppm sodium hypochlorite and subsequently dechlorinated by aeration for 48 h, before adding to the tanks. During the stunting phase, 80% water exchange was carried out daily in the morning (09,00 hrs). During the post-stunting period, water exchange was reduced to 30% per day for both the treatments. Continuous aeration was provided from a 5 HP blower, with a single aeration stone placed at the centre of each tank to ensure sufficient dissolved oxygen levels in the water throughout the experimental period. Water samples were collected weekly from each tank, prior to water exchange, for water quality analysis. The initial average weight of the fish was recorded prior to stocking in experimental tanks. Growth sampling was done fortnightly for recording average body weight and adjust feed rations accordingly. The daily feed ration was divided and administered in the morning at 10.00 hrs and in the evening at 17.00 hrs. The uneaten feed and bottom debris were removed the following morning by siphoning. Individual fish were measured for length and weight, for morphometric analysis. A commercial feed (Nutrila, Growel India Pvt. Ltd.) of particle size ranging from 0.8 to 1.2 mm and containing 45% crude protein; 10% crude fat; 2.5% crude fibre and 11% moisture was used for the experiment.

Experimental rearing

Snubnose pompano fingerlings (approximately 4 cm in length), were procured from the marine finfish hatchery of the Mandapam Regional Centre of ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Mandapam, Tamil Nadu, India. During the stunting phase, fish were stocked, at a stocking density (SD) of 60 fish per tank in triplicate, fed at 3% of average body weight (ABW) and reared for 30 days. Similarly, a normal group was maintained in three separate FRP tanks at a lower stocking density of 20 fish per tank and fed at 10% of ABW during the same period. Following the stunting phase, the stunted fish were further reared for an additional 30 days during the post-stunting phase. During this phase, the stunted fish were restocked at a lower density of 20 fish per tank and fed at approximately 15% of ABW.

Growth and biometry

During each growth sampling, 15 fish were randomly sampled from each group to record individual length and weight. Total length of the fish, measured from the tip of the snout to the tip of the caudal fin was measured using a 1 m wooden scale with an accuracy of 1 mm. Total body weight was measured using an electronic weighing balance (Sartorius, Germany) with an accuracy of 0.001 g. Specific growth rate (SGR) per day was calculated according to De Silva and Anderson (1995) as: Specific growth rate (% per day) = [In (Final weight) - In (Initial weight)] / [Experimental days] x 100. where 'ln' is the natural logarithmic value. Condition factor (k) was determined as per Gomiero and Braga (2005) using the formula: $k = (W \times 100) / L^3$, where k = Condition factor; W = Weight of the fish in gram (g) and L= Total length of the fish in centimetres (cm). At the end of the experiment, the number of surviving fish in each tank were counted and the survival rate (%) was calculated as Survival (%) = (Total number of fish survived x 100) / Total number of fish stocked. The length-weight relationship was derived from the log-transformed total body length and body weight data using the equation: Log (Weight) = $a + [b \times log (Length)]$ where 'a' is the intercept and 'b' is the slope of the linear regression on the log transformed weight (g) and length data (cm). Pooled samples from all replications of each treatment were used for this analysis. Hepato-somatic Index (HSI) was calculated as: HSI (%) = (Weight of liver x 100) / Weight of fish.

Proximate composition of fish carcass

The whole-body nutrient composition of fish carcass was assessed at the end of both the stunting and post-stunting phases, following standard methods (AOAC, 1995). For this, three fish from each treatment were selected. Their wet weight was recorded and the samples were dried in a hot air oven at 105°C overnight until a constant dry weight was achieved. Crude protein (N x 6.25) was determined by the Kjeldhal method (FOSS Kjeltec, 2300). Crude lipid was determined by the ether extraction method using a Soxhlet system (FOSS Soxtec, 2043). Ash content was determined by incinerating the samples in a muffle furnace at 550°C. For determination of acid-insoluble ash content, the ash was boiled with dilute hydrochloric acid (HCI), filtered, and the residue was re-ignited and the remaining acid-insoluble fraction was weighed. Crude fibre content was estimated following AOAC (1995). All nutrient concentrations were calculated as percentage based on dry weight.

Evaluation of protein levels in various tissues

At the end of the stunting and post-stunting rearing, three fish from each experimental replication, were anesthetised using benzocaine at a concentration of 10 ppm for a duration of 3 min. Following anesthesia, blood samples were collected *via* caudal vein puncture using sterile syringes. The blood samples were allowed to clot at room temperature for 1 h and then refrigerated overnight at 4°C. Subsequently, the samples were centrifuged at 6,000 rpm for 10 min to separate the serum. The serum from each replicate was pooled according to the respective treatment and stored at -20°C until further analysis. The gill and liver tissues were collected aseptically from the dissected fish and the tissues were homoginised in 0.25 M sucrose containing 1 mM EDTA and centrifuged at 6,000 rpm for 10 min. The resulting homoginates were stored at -20°C until further analysis.

The total protein concentration in serum samples was determined using the Biuret method. In this assay, the proteins react with cupric ions in an alkaline medium to form a blue-violet complex, the intensity of which is directly proportional to the protein concentration. The absorbance of the resulting complex was measured spectrophotometrically at 550 nm. For the total protein analysis of tissues, the Lowry's method was employed using a commercial kit (Genie, Bangalore) following the manufacturer's instructions.

Water quality analysis

Water quality parameters, including dissolved oxygen, pH, temperature, salinity and ammonium (NH_4^+) concentration, were monitored at weekly intervals following standard procedures (APHA, 1981).

Statistical analysis

Statistical significance for all tested parameters was at p<0.05. The mean values were compared between stunted and normal fish using t test to determine significant differences (p<0.05). Results expressed as ratios or percentage were not subjected to t test, in accordance with our previous report (Anikuttan *et al.*, 2021).

Results

Water quality

Water quality parameters during the rearing of stunted fish are presented in Fig. 1. Total ammonia levels ranged from 0.2 and 1 ppm, while pH values varied between 8 to 8.2. Both total ammonia and pH were consistently higher in the tanks containing stunted fish, compared to those with normal fish. Dissolved oxygen levels ranged from 3 to 5 ppm and were mostly lower in the stunted fish tanks. During the stunting period, water temperature ranged from 27 to 28°C.

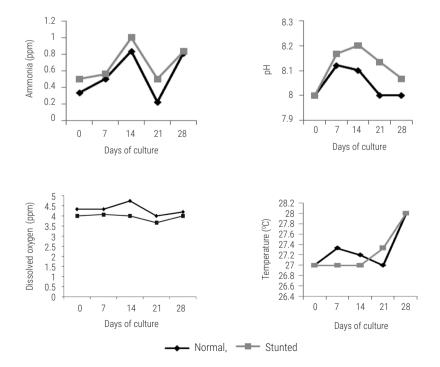


Fig. 1. Comparison of water quality parameters in tanks with stunted and normal T. blochii during the stunting stage

Observations during the stunting phase

A comparison of the growth performance, proximate composition of meat and total protein content in different tissues of stunted fish and normal fish at the end of stunting period is given in Table 1. The morphometric data revealed that normal fish had significantly higher final ABW (5.4±0.23 g) and final length (6.18±0.077 cm) compared to stunted fish. Throughout the experimental period, stunted fish exhibited marked growth retardation, attaining significantly lower length and weight in comparison to the normal fish (Fig. 2; Table 1).

The effect of short-term stunting on biometric parameters such as length-weight relationship (LWR) and condition factor was studied.

Table 1. Growth, survival, length-weight relationship, proximate composition of carcass and protein content in various tissues of stunted and normal *T. blochii*

Parameters	Normal fish	Stunted fish
Growth and survival		
Initial length (cm)	4.2 ± 0.083	4.2 ± 0.083
Final length (cm)	6.18 ± 0.077ª	5.67 ± 0.2 ^b
Initial weight (g)	2.2 ± 0.38	2.2 ± 0.38
Final weight (g)	5.4 ± 0.23ª	4.46 ± 0.18^{b}
Specific growth rate (% per day)	2.99 ± 0.15ª	2.31± 0.13b
Survival (%)	96.66	88
Length-weight relationship		
Number of fish	30	30
Slope (b)	2.24	2.58
Intercept	2.44	3.06
R ²	0.81	0.87
Condition factor	2.28± 0.02ª	2.47 ± 0.27ª
Proximate composition of meat (g p	er100 g on dry weig	ght basis)
Crude protein	57.59±0.90ª	55.81±0.42ª
Crude lipid	18.12±1.14ª	15.5±0.81 ^b
Crude fibre	0.02±0.008ª	0.02±0.01ª
Total ash	10.64±0.20ª	10.22±0.08ª
Acid insoluble ash	1.54±0.04ª	1.07±0.89b
Hepato-somatic Index (%)	3.6±0.47ª	5.18±0.53 ^b
Total protein in different tissues		
Gill (%)	4.47±0.57ª	2.60±0.35b
Liver (%)	5.28±0.59ª	10.34±0.001b
Serum (%)	6.81±0.16ª	6.64±0.001ª

LWR fit for both stunted and normal fish was significant with R² values greater than 0.8, indicating a good fit. Both groups exhibited negative allometric growth patterns (b<3). Growth in terms of SGR (%) per day was significantly lower (p<0.05) in stunted fish, confirming growth retardation. However, the condition factor did not differ significantly (p>0.05) between the two treatments. However, stunted fish had a slightly higher condition factor (2.47±0.27) compared to the normal fish. The HSI (%) was significantly higher in stunted fish than normal fish.

The proximate composition of fish carcass (Table 1) indicated that normal fish had a significantly higher (p<0.05) percentage of crude lipid and acid insoluble ash compared to stunted fish. Although crude protein and total ash levels were slightly higher in normal fish, the differences were not statistically significant (p>0.05). Crude fibre levels were comparable between the two groups.

Total protein content in different organs is presented in Table 1. In stunted fish, total protein levels in the gills were significantly lower, while total protein in liver was significantly higher compared to normal fish. However, no significant difference was observed in serum protein levels between the two groups.

Observations during the post-stunting phase

A comparison of the growth, proximate composition of meat and, total protein content in different tissues of stunted fish at the end of the stunting and post-stunting periods are presented in Table 2. Post-stunted fish exhibited increases in both final length and weight, indicating compensatory growth. However, SGR (% per day) remained low, during this period suggesting only partial compensatory growth. Crude lipid levels in both post-stunted fish was significantly (p<0.05) lower than that in stunted fish. In contast, crude protein, crud fibre, total ash content and acid insoluble ash contents did not differ significantly between the post-stunting and stunting periods. During the post-stunting period, the HSI (%) showed a significant decrease in post-stunted fish compared to the stunted fish. Total protein in gill has increased significantly (p<0.05) during the post-stunting period, while liver total protein content decreased drastically (p<0.05) in post-stunted fish. Serum protein levels remained relatively similar in the stunted fish during the stunting and post-stunting periods.

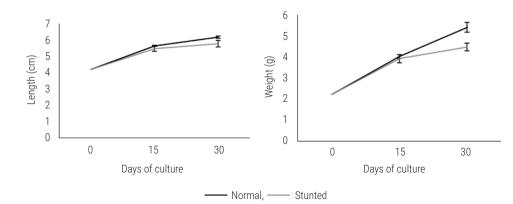


Fig. 2. Comparison of increment in length and weight in stunted and normal T. blochii during the stunting stage

Table 2. Comparison of growth, survival, length-weight relationship, proximate composition of carcass and protein content in various tissues in stunted and post-stunted *T. blochii*

Parameters	Stunted fish	Post-stunted fish
Final length (cm)	5.67 ± 0.2ª	8.68 ± 0.05 ^b
Final weight (cm)	4.46 ± 0.18ª	8.12 ± 0.16 ^b
Specific growth rate (% per day)	2.31± 0.13ª	2.01± 0.2 ^b
Proximate composition of meat (g pe	r 100 g on dry w	eight basis)
Crude protein	55.81±0.42ª	56.04±0.02ª
Crude lipid	15.5±0.81ª	11.87±0.02 ^b
Crude fibre	0.02±0.01ª	0.02±0.006ª
Total ash	10.22±0.08ª	9.46±0.11ª
Acid insoluble ash	1.07±0.89ª	0.90±0.68ª
Hepato-somatic Index (%)	5.18 ± 0.53ª	1.5 ± 0.31 ^b
Total protein		
Gill (%)	2.60±0.35ª	4.39±0.97b
Liver (%)	10.34 ± 0.001ª	2.66 ± 0.15ª
Serum (%)	6.64±0.001ª	6.04±0.62ª

Discussion

Observations during the stunting phase

The stunted fish grew slower than normal fish during the stunting period, with significantly lower length, weight and SGR (%) per day (p<0.05) indicating that the method used to produce stunted fish was proper for growth retardation. Suresh Babu *et al.* (2022) and Anikuttan *et al.* (2021) found similar growth patterns in snubnose pompano stunted in indoor tanks. Since the stunting period was short, the difference in length and weight between stunted and normal fish was less. Groat (2002) reported similar findings, stating that Florida pompano (*Trachinotus carolinus*) grew faster when fed up to satiation rather than at 5% body weight per day in closed recirculating systems. According to the findings of Hamed *et al.* (2016), *T. blochii* fed at a 10% body weight per day, gain more weight than those fed at a lower rate of 3% body weight per day.

The specific growth rate of stunted fish (2.3% per day) was found to be significantly lower than that of normal fish (2.99% per day) owing to retarded growth. Chavez et al. (2011) reported SGR of 2.63 - 2.72% per day in T. blochii, fed at 4 - 10% of body weight in 120 days in marine floating cages and Suresh Babu et al. (2022a) reported 2.1% in stunted fish and 2.7% in normal fish. Hamed et al. (2016) found a similar SGR trend in T. blochii, when fed at 3 - 10% of body weight per day. SGR of about 2% per day was reported by Jayakumar et al. (2014) and Kalidas et al. (2012) from brackishwater pond and indoor tanks respectively. However, Suresh Babu et al. (2022b) and Damodaran et al. (2019) reported lower SGR in recirculating aquaculture systems and pond culture of the same species. It is found that 3% ABW per day feeding is sufficient for stunting juvenile silver pompano, because growth was retarded at this feeding rate, which is comparable to previous reports in T. blochii (Suresh Babu et al. 2022a) and other tropical fish such as Chanos chanos (1% body weight per day; Lingam et al., 2019).

The impact of stunting on biometric parameters such as the LWR and condition factor, at the end of stunting period was analysed. In the present study, the LWR showed a good fit for

both stunted and normal fish, with R² values greater than 0.8. Both groups exhibited negative allometric growth pattern (b<3). Similar hypo-allometric growth in pond grown silver pompano, was reported by Damodaran *et al.* (2019), consistent with the findings of the present study. According to Cherif *et al.* (2008), a higher b value in the LWR indicates greater growth potential at a given length. The relatively lower b values observed in this study could be attributed to the limitation of the indoor tank culture conditions.

In the current study, condition factor (K) did not differ significantly between stunted and normal fish and was greater than 2 in both groups, indicating that the fish remained in good physiological condition. Similarly, Suresh Babu *et al.* (2022 a, b) and Damodaran *et al.* (2019) reported condition factor values greater than one for silver pompano grown in RAS, low saline cages and pond conditions respectively. Condition factor (K) is a widely used indicator of fish health and body condition and it has been shown to influence body composition of the fish (Ali *et al.*, 2003).

Farming stunted fish alters the proximate composition of the fish due to differences in nutrient utilisation during the stunting phase (for the metabolism) which may be regained depending on the degree of compensation (Jobling, 2010; Lingam et al., 2019). When the proximate composition of meat from stunted and normal fish was compared, stunted fish had significantly lower crude lipid and acid insoluble ash. Crude protein, crude fibre and total ash did not differ significantly (p>0.05). Luo et al. (2009) reported that in channel catfish Ictalurus punctatus stocked in indoor flow-through fiberglass tanks and starved for 80 days, both protein and lipid levels decreased, and changes in the amount of fat were relatively rapid. According to Hamed et al. (2016), carcass proximate composition of T. blochii fed at higher feeding rates had significantly higher lipid and an associated decrease in moisture, protein, and ash content compared to lower feeding rate. According to Bureau et al. (2006) fish fed at lower feeding levels had positive protein deposition, but had negative lipid deposition, implying that fish fed at low levels mobilise body lipid reserves to support protein deposition. Bhujel et al. (2007) reported a lower percentage of carcass fat in stunted Nile tilapia, fed at 3% of body weight than the normal fish, in support of the present study. Lingam et al. (2019) also reported a significant reduction in carcass fat content in stunted milkfish compared to normal fish

Stunted fish had a significantly higher hepato-somatic index (%) than normal fish. As starvation progressed, hepatic lipid and carbohydrate contents decreased, but crude protein increased (p<0.05) indicating the deposition of protein than lipid in the liver. Jobling (1980) found that short-term starvation in plaice, *Pleuronectes platessa*, significantly reduced the fat content. In addition to this, Quinton and Blake (1990), also, reported that starvation significantly reduced the fat and increased the moisture in rainbow trout. In stunted fish, total protein in the gills was significantly lower, while the total protein in liver was significantly between the two groups. Eslamloo *et al.* (2017) stated that plasma protein was used only as a fourth energy source after the depletion of stored energy reserves of glycogen, glucose and lipid. In fish, lipids are the main energy source and are broken down early in the fasting phase (Ali *et al.*, 2003).

Impact of stunted fish production on water quality

Dissolved oxygen level ranged from 3 to 5 ppm and was lower in the stunted fish tanks, since stocking density (SD) was higher in these tanks. During the stunting period, the temperature ranged from 27 to 28°C. Water quality parameters such as dissolved oxygen and temperature were within the range for snubnose pompano farming in low saline conditions. Javakumar et al. (2014) reported similar ranges in low saline pond conditions and Suresh Babu et al. (2022) reported similar values in indoor experiments. However, the total ammonia and pH ranges were 0.2 to 1 ppm and 8 to 8.2, respectively. In an indoor stunting experiment of snubnose pompano in marine conditions, Anikuttan et al. (2021) reported a total ammonia range of 0.1 to 0.3 ppm. In stunted fish tanks, total ammonia and pH were always greater than in normal fish tanks, which could be due to higher SD in these tanks. These results reveal that overcrowding during stunting influence the water quality of the system and the same need to be optimised employing biological filtration systems. Enhanced ammonia level, reduced dissolved oxygen and the crowding stress imparted lower survival (%) in stunted fish.

Observations during post-stunting phase

The final length and weight of fish increased during post-stunting in the stunted fish, indicating compensatory growth. But the specific growth rate of post-stunted fish is significantly lower than that of stunted fish indicating partial compensatory growth at this point and the growth may compensate if reared for a longer period. Anikuttan et al. (2021) and Suresh Babu et al. (2022a) also reported enhanced growth in stunted fish with partial compensation and moderate specific growths, compared to normal fish. Compensatory growth in the post-stunting phase causes variations in morphological, muscular and carcass composition, which affect the nutritional quality and processing efficiency of the post-stunted fish. The most notable quality changes in post-stunted fish were observed in the proximate composition of the carcass. Only minor difference in biochemical composition of the meat was observed between post-stunted and stunted fish, but with significantly lower lipid content. This implies that lipid reserve is utilised for the compensation of growth during the post-stunting phase. Lingam et al. (2019) also reported a lower carcass fat content in post-stunted milkfish compared to normal fish. Jobling et al. (1994) stated that in the compensatory growth phase, post-stunted fish tend to deposit more lean body mass (particularly protein), in the tissues which, sequentially increases the protein content of muscle and carcass. During the post-stunting phase, the HSI (%) in stunted fish decreased significantly due to maximum utilisation of reserve nutrients for compensatory growth. The lower lipid content in the muscle and the lower HSI indicate that mainly fat reserves are utilised for the growth compensation and a protein sparing action in the post-stunting phase.

The present work indicates that short term stunting (for one month) can induce retarded growth in silver pompano which can be compensated when the favourable conditions are restored. Short term stunting is having only negligible impact on the morphometric indices such as length-weight relationship and condition factor. Also, the proximate analysis indicates that lipid metabolism plays a major role in the stunting and compensatory growth of the fish. Also, the study reveals that overcrowding during stunting influence the water quality of the system and the same need to be optimised employing biological filtration systems.

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