Nursery rearing of Asian seabass (*Lates calcarifer* Bloch, 1790) fry in indoor recirculatory aquaculture system

MOHAMED FAIZULLAH[1*], MUTHU ABISHAG[1] and SETHURAJ ARCHANA[2]

[1]Dr MGR Fisheries College and Research Institute
Tamil Nadu Dr J Jayalalithaa Fisheries University
Thalainayeru 614712 Tamil Nadu, India
[2]Fisheries College and Research Institute, Tamil Nadu Dr J Jayalalithaa Fisheries University
Thoothukudi 628008 Tamil Nadu, India
[*]Email for correspondence: faizullahmfsc@gmail.com

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ABSTRACT

The present study evaluated the effect of different stocking densities on the growth performance, cannibalism and survival of Asian seabass (*Lates calcarifer*) fry reared in an indoor recirculating aquaculture system (RAS). Fry (25 days post-hatch) were stocked at three densities: 100 (T_1), 200 (T_2), and 300 (T_3) individuals per m^3 and reared for 50 days under controlled conditions. Growth parameters, water quality, cannibalism rate and survival were monitored throughout the study. Water quality parameters remained within acceptable limits across treatments, although slight variations were observed with increasing density. Fish reared at the lowest density (T_1) exhibited significantly higher growth performance, with maximum length gain (8.40 ± 0.95 cm), weight gain (10.2 ± 0.16 g) and better feed conversion efficiency. In contrast, higher stocking densities (T_2 and T_3) resulted in reduced growth and feed efficiency. Cannibalism rate increased significantly with density, reaching the highest value in T_3 (13.55 ± 2.84%), while survival rate decreased from 96.54 per cent in T_1 to 86.45 per cent in T_3. The results indicated that lower stocking density improves growth performance, reduces cannibalism and enhances survival in Asian seabass fry under RAS conditions. Optimizing stocking density is therefore critical for sustainable and efficient nursery rearing of seabass.

Keywords: Asian seabass; *Lates calcarifer*; Recirculating aquaculture system (RAS); Stocking density; Growth performance; Cannibalism; Survival rate; Nursery rearing

INTRODUCTION

Asian seabass (*Lates calcarifer*), commonly known as Barramundi or giant perch, is a commercially important food fish belonging to the family Latidae. It is widely distributed across the Indo-West Pacific region, including the Arabian Gulf, India, China, Taiwan, Papua New Guinea and Australia (Anon 2009). The species is highly regarded for aquaculture due to its strong market demand, rapid growth rate, tolerance to high stocking densities, adaptability to diverse environmental conditions, high fecundity, and readiness to accept artificial feed. Commercial farming of Asian seabass was first successfully established in Thailand during the 1970s and later expanded to Australia in the 1980s (Mathew 2009). The nursery rearing phase typically lasts 30-45 days, during which fry grow to a size of 8-12 cm (Ayson et al 2014). Owing to its euryhaline nature and adaptability, seabass can be cultured in a wide range of production systems, including ponds, stagnant water bodies, dugout tanks, floating net cages, raceways and recirculating aquaculture systems (RAS) (Philipose et al 2010). Among these, RAS is increasingly recognized as a climate-resilient and sustainable aquaculture system due to its efficient water use, environmentally friendly operation and capacity to maintain controlled rearing conditions with minimal climatic interference. However, elevated stocking densities in RAS can act as a significant stressor, negatively impacting growth (Lefrançois et al 2001) and survival (Rowland et al 2006). Additionally, as an opportunistic carnivore, Asian seabass exhibits

increased cannibalistic behaviour under high-density conditions (Mojjada et al 2013, Manley et al 2014). Juveniles, characterized by rapid growth and high feeding activity, often display considerable size heterogeneity, which further promotes cannibalism (Baras and Jobling 2002). This behaviour is particularly severe during the nursery phase and can result in substantial losses before the fish reach a length of 10 cm (Qin et al 2004). Although size grading is currently the most effective strategy to mitigate cannibalism, it is labour-intensive, stressful to fish and may increase mortality risk. In this context, the present study was undertaken to evaluate the effects of different stocking densities on growth performance, cannibalism and survival of Asian seabass (*L calcarifer*) fry reared in a recirculating aquaculture system.

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MATERIAL and METHODS

Experimental design

The study was conducted in an indoor RAS facility at the Freshwater Instructional Fish Farm, Korukkai, Tamil Nadu. Six indoor circular fibreglass reinforced plastic (FRP) tanks with central drainage, each having a water holding capacity of 1,000 L, were used in the system. The filtration unit consisted of an FRP biofilter with four partitions of 2 m³ capacity (200 cm length × 90 cm width × 100 cm height), fitted with a 63 mm diameter inlet and a 90-75 mm diameter outlet. The four partitions contained oyster shells (0.5 m³), bio-wheels (1 m³), gravel (0.4 m³) and activated charcoal (0.1 m³) for biofiltration of wastewater. In addition, a fibre bubble wash bead filter with 3 mm bead media and backwash facility for solids removal, along with a fibreglass skimmer of 5-ton flow rate and a 40 cm diameter venturi system for foam removal, were incorporated into the system. A 1 HP pump was used for water circulation and the flow rate was maintained at 5 L per min (Fig 1, Plate 1). Water was continuously aerated to saturation (5-6 ppm) using two air blowers (flow rate = 18,000 L/h) connected to each tank. *L calcarifer* fry (25 days post-hatch) were procured from the Rajiv Gandhi Centre for Aquaculture, Sirkazhi, Tamil Nadu. The average initial weight and total length of the fry were 0.2 ± 0.42 g and 20 ± 4.84 mm respectively. The fry were packed in polythene bags (salinity: 5‰, pH: 8.0, temperature: 28.5°C), transported and acclimatized in 1,000 L FRP tanks at three stocking densities viz 100, 200 and 300 per m³, designated as T_1, T_2 and T_3 respectively. Acclimatization was carried out for 2 days. During this period, the fish were fed daily with Growel® Nutrila floating feed (crude protein 52%, crude lipid 12%, crude fibre 1.5%, ash 18% and moisture 12%). The daily ration was fixed at 10 per cent of the body weight of the fish. As inadequate nutrition may lead to cannibalistic behaviour in Asian seabass juveniles, fish were fed four times a day at 7:00 am, 12:00 pm, 5:00 pm and 10:00 pm. Uneaten feed and faecal matter were siphoned out one hour after each feeding. Water circulation was temporarily stopped during feeding and resumed after the removal of waste.

Growth parameters (fish grading and fish sampling)

The study was conducted for 50 days. At the start of the experimental trial a sample of 10 seabass fry was used to measure total body length and mean weight. Seabass fry were stocked at densities of 100, 200 and 300 per m³ in different RAS tanks and growth performance was monitored over a 50 day period. The fish were graded every 10 days using a manual grader and the shooters were reared in separate tanks. After grading representative samples were randomly collected using a scoop net for the analysis of growth parameters. The growth parameters were determined following the methods outlined by Salama and Al-Harbi (2007):

$$\text{Length gain (cm)} = \text{Final length} - \text{Initial length}$$

$$\text{Weight gain (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{Specific growth rate (SGR) (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Days}} \times 100$$

$$\text{Feed conversion ratio (FCR) (\%)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}} \times 100$$

$$\text{Cannibalism rate (\%)} = \frac{\text{Number of fry lost to cannibalism}}{\text{Total number of fry}} \times 100$$

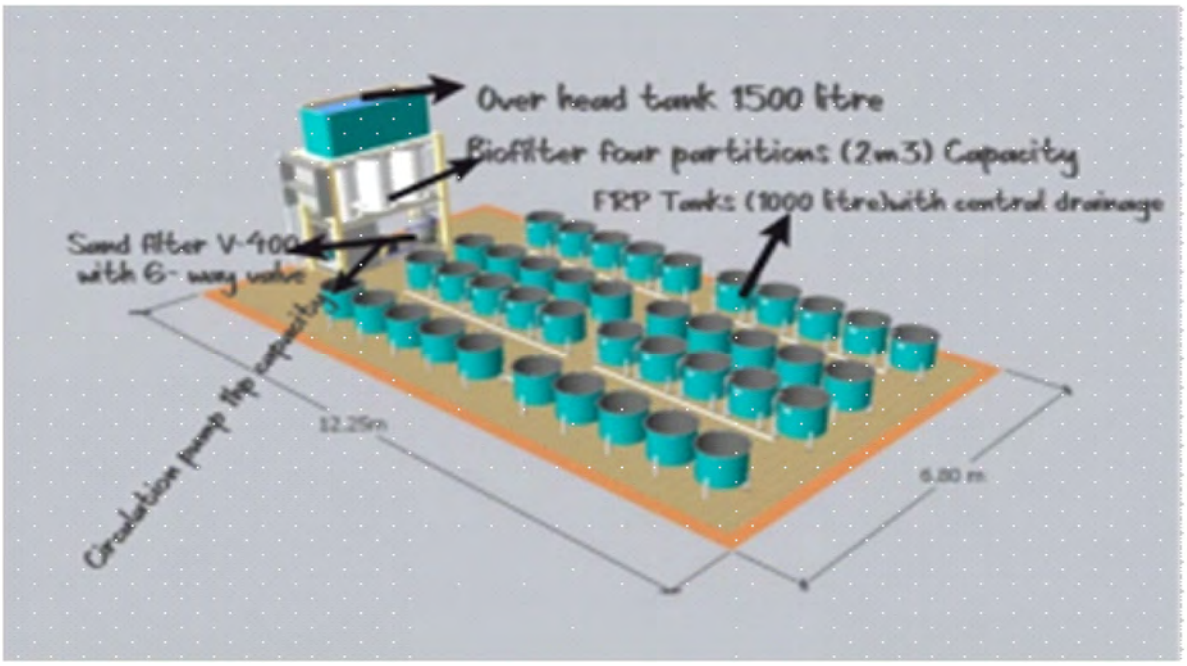

Fig 1. Design and layout of recirculatory aquaculture system used in the experiment



Plate 1. Construction of the recirculatory aquaculture system used in the experiment

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Water quality parameters

Throughout the study period key water quality parameters including temperature dissolved oxygen and pH were monitored twice daily at 9:00 AM and 7:00 PM in the culture systems. Water temperature was measured using a thermometer with 0.1°C precision. A laboratory grade pH meter (Systronic-362 India) was used to determine pH levels. Dissolved oxygen concentration was estimated by the modified Winkler titration method. Additional parameters such as total alkalinity ammonia (NH*f*) and nitrite (NO,) were assessed twice weekly using titration methods as described by Anon (1980).

Feeding

The fish were fed 1 mm diameter commercial semi-floating pellet feed (52% crude protein) at 10 per cent of their biomass, distributed six times daily at 6:00

am, 10:00 am, 2:00 pm, 6:00 pm, 10:00 pm and 2:00 am during the first 20 days. For the next 25 days the fish were fed 2 mm diameter commercial floating pellet feed (45% crude protein) while the feeding rate and frequency were maintained constant. Water circulation was temporarily stopped during each feeding period. Uneaten feed and faecal matter were siphoned out one hour after feeding after which water circulation was resumed. The feed was rapidly consumed with no pellets remaining at the bottom.

Statistical analysis

All analyses were carried out in duplicate. The data obtained in the study were analysed using analysis of variance (ANOVA). Significance levels of $P < 0.05$ and $P < 0.01$ were used for validation (Snedecor and Cochran 1989). Differences among treatments were further analysed using Tukey's post hoc test as per Kim (2015).

RESULTS and DISCUSSION

Water quality parameters

In the present study, water quality parameters demonstrated observable trends in response to increasing stocking densities within the RAS, which may have implications for fish health and growth (Table 1).

Similar patterns of water quality fluctuation with stocking density have been reported in previous studies, reinforcing the need to balance stocking density with optimal water quality management for maximizing fish welfare and performance. Temperature values showed a small but consistent increase with stocking density, from 29.0 ± 0.42°C at T_1 to 29.4 ± 0.39°C at T_3. However, no statistically significant differences could be observed ($p > 0.05$).

This increase aligns with findings by Ellis et al (2005), who noted that higher stocking densities often contribute to greater metabolic activity and heat production within the system, potentially elevating temperature. However, this small rise in temperature remained within acceptable ranges for seabass, as reported by Hassan et al (2021), suggesting that the slight variations observed are unlikely to negatively impact growth rates or health.

Dissolved oxygen (DO) levels demonstrated a minor decline with higher densities, which was recorded at 7.5 ± 0.14 mg per L for T_1 and 7.3 ± 0.10 mg per L for T_3 and the mean values were statistically significant ($p < 0.05$).

While this reduction is consistent with findings by Yang et al (2024), who also observed decreasing DO levels at higher densities due to elevated oxygen consumption from increased biomass, it remained within the acceptable range for seabass culture. However, studies such as those by Ali et al (2022) have emphasized that long-term reduction in DO levels, even if slight, could lead to suboptimal growth or increased susceptibility to stress in high-density aquaculture systems.

The pH levels increased slightly as stocking density rose, with values of 8.0 ± 0.04, 8.1 ± 0.05 and 8.2 ± 0.06 for T_1, T_2 and T_3 respectively and the values were statistically significant ($p < 0.05$). Similar increases

Table 1. Effect of different stocking densities on the water quality parameters of Asian seabass fry in indoor recirculating aquaculture system (RAS)-based nursery

Water quality parameter	Stocking density		
	T_1 (100/m^3)	T_2 (200/m^3)	T_3 (300/m^3)
Temperature (°C)	29.0 ± 0.42^a	29.2 ± 0.40^a	29.4 ± 0.39^a
Dissolved oxygen (mg/L)	7.5 ± 0.14^a	7.4 ± 0.12^a	7.3 ± 0.10^b
pH	8.0 ± 0.04^c	8.1 ± 0.05^b	8.2 ± 0.06^a
Salinity (ppt)	4.1 ± 0.12^a	4.2 ± 0.14^a	4.3 ± 0.14^a
Total alkalinity (mg/L)	156 ± 2.14^c	162 ± 1.98^b	168 ± 2.25^a
NH_3 (mg/L)	0.012 ± 0.00^a	0.014 ± 0.01^a	0.018 ± 0.03^a
NO_2 (mg/L)	0.80 ± 0.09^a	0.92 ± 0.12^a	0.98 ± 0.18^a

Results expressed as mean ± standard deviation, Mean values with different superscripts in the same row are statistically significant ($p < 0.05$)

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in pH at higher stocking densities were observed by Sammouth et al (2009), who attributed this to the higher organic matter breakdown and subsequent alkalinity production. While the pH values recorded in this study remained within the ideal range for seabass, as supported by research from Noval et al (2019) continued monitoring had prevented any shifts beyond the optimal range, which could have stressed the fish.

Salinity also exhibited a minor increase across the densities, measured at 4.1 ± 0.12, 4.2 ± 0.14 and 4.3 ± 0.14 ppt for T_1, T_2 and T_3 respectively. However, no statistically significant differences could be observed ($p > 0.05$). This trend, although minor, is consistent with observations by Zhang et al (2011) who also found that increased biomass can slightly elevate salinity levels due to water evaporation and concentration effects in RAS environments.

However, since these salinity values fall within the appropriate range for seabass culture (Wijayanto et al 2021), they are unlikely to adversely affect fish growth or physiology. Additionally, total alkalinity increased with density, ranging from 156 ± 2.14 mg per L at T_1 to 168 ± 2.25 mg per L at T_3 and the values were statistically significant ($p < 0.05$).

Total alkalinity values are consistent with the findings of Boyd et al (2016), who reported that higher densities tend to increase alkalinity through greater excretion rates and organic material decomposition. Elevated alkalinity can serve as a buffer against pH fluctuations. Timmons and Ebeling (2013), potentially contributing to system stability at higher densities, although excessive levels should be carefully managed to avoid potential imbalances.

Ammonia (NH_4) and nitrite (NO_3) levels increased with stocking density, as expected, given that higher stocking densities lead to greater nitrogenous waste production (Ebeling et al 2006). Ammonia concentrations, although low, rose from 0.012 ± 0.00 mg per L at T_1 to 0.018 ± 0.03 mg per L at T_3, while nitrite increased from 0.80 ± 0.09 mg per L to 0.98 ± 0.18 mg per L over the same density range. While these values remained below harmful thresholds (Timmons and Ebeling 2013), it is crucial to continue monitoring these parameters to avoid potential toxicity. Excessive accumulation of ammonia and nitrite can impair fish growth and health, as suggested by Hu et al (2019) and may necessitate enhanced filtration or water exchange protocols at higher densities. Hence, though increased stocking densities in RAS led to minor changes in water quality parameters, these remained within safe limits for seabass culture. Nevertheless, continuous monitoring and management are essential in maintaining optimal conditions, particularly in high-density settings, to ensure sustainable fish growth and welfare Liu et al (2017).

Growth parameters

The impact of different stocking densities on the growth performance and survival of fish reared in RAS is provided in Table 2.

Table 2. Effect of different stocking densities on the growth performance, cannibalistic behaviour and survival rate of Asian seabass fry in indoor recirculating aquaculture system (RAS)-based nursery

Growth parameter	Stocking density		
	T_1(100/m^3)	T_2(200/m^3)	T_3(300/m^3)
Final total length (cm)	10.4 ± 0.52^a	9.2 ± 0.46^{ab}	8.8 ± 0.42^b
Final total weight (g)	11.4 ± 0.61^a	9.6 ± 0.51^{ab}	9.2 ± 0.47^b
Length gain (cm)	8.40 ± 0.95^a	7.2 ± 0.05^b	6.8 ± 0.07^c
Weight gain (g)	10.2 ± 0.16^a	8.4 ± 0.11^b	8.0 ± 0.11^c
Specific growth rate (%)	4.77 ± 1.05^a	4.41 ± 0.95^a	4.31 ± 0.89^a
Food conversion ratio (FCR)	2.67 ± 0.04^a	2.74 ± 0.06^b	2.83 ± 0.09^b
Cannibalism (%)	3.46 ± 1.56^b	10.36 ± 2.36^a	13.55 ± 2.84^a
Survival rate (%)	96.54 ± 1.56^a	89.64 ± 2.36^b	86.45 ± 2.84^b

Results expressed as mean ± standard deviation, Mean values with different superscripts in the same row are statistically significant ($p < 0.05$)

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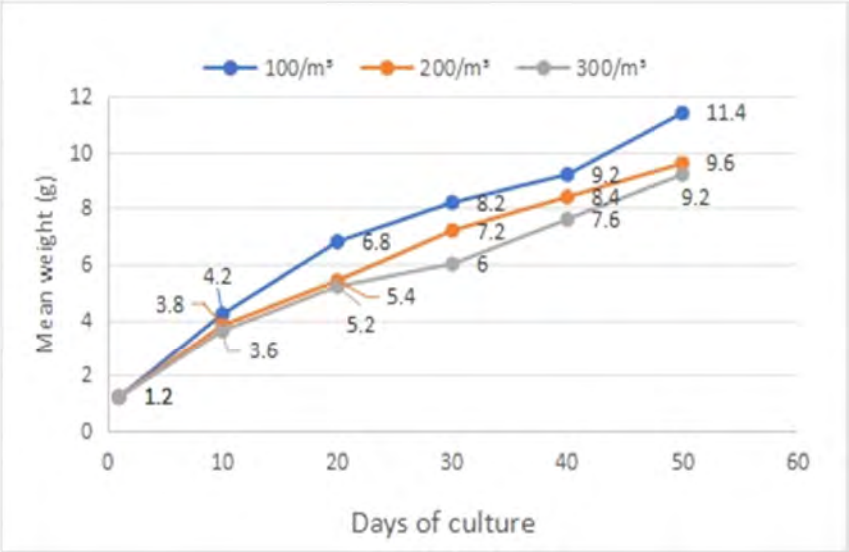

Fig 2. Effect of different stocking densities on the growth increment of Asian seabass in RAS system

The growth parameters exhibited significant differences ($P < 0.01$) over the period of culture and among the stocking densities in the nursery rearing of seabass (Fig 2). However, length and weight gain were significantly different with respect to both days of culture and the stocking densities ($P < 0.05$). Fish at T_1 exhibited the highest final total length (10.4 ± 0.52 cm) and weight (11.4 ± 0.61 g), along with the greatest length gain (8.40 ± 0.95 cm) and weight gain (10.2 ± 0.16 g). In contrast, at higher stocking densities of T_2 and T_3, the final total length and weight were significantly reduced, measuring 9.2 ± 0.46 cm and 9.6 ± 0.51 g and 8.8 ± 0.42 cm and 9.2 ± 0.47 g respectively. This aligns with the findings by Lindholm-Lehto (2023) and Ganesh et al (2023) who also observed that lower stocking densities allow for enhanced individual growth in seabass and other species due to reduced competition for space and resources.

Increased growth at lower densities can be attributed to reduced stress, which enhances feeding efficiency and nutrient assimilation (Salari et al 2012). Conversely, higher densities often restrict fish movement, increase metabolic waste production and elevate stress levels, ultimately leading to suboptimal growth performance (Mladineo et al 2010).

Additionally, a gradual decline in specific growth rate (SGR) was observed with increasing stocking density, with values of 4.77 ± 1.05 per cent at T_1, 4.41 ± 0.95 per cent at T_2 and 4.31 ± 0.89 per cent at T_3. However, no statistically significant differences could be observed ($p > 0.05$) among the treatments. This is consistent with studies by Khan et al (2021); Hassan et al (2024) who found that SGR tends to decrease as fish density increases due to competition for feed and limited access to resources.

T_1 exhibited the highest SGR, while T_3 had the lowest, highlighting the adverse effects of overcrowding on growth efficiency. Similarly, the food conversion ratio (FCR) increased with stocking density, indicating poor feed efficiency at higher densities. These findings mirror those reported by Khan et al (2021) where higher densities in RAS systems resulted in reduced feed utilization efficiency due to heightened competition and stress.

Cannibalism and survival

The observed increase in cannibalism with rising stocking density further highlights the behavioral impacts of crowding. Cannibalism rates surged from 3.46 per cent at T_1 to 13.55 per cent at T_3 ($p < 0.05$), a pattern that has been similarly reported in other studies, such as Baras and Jobling (2002), which emphasized the role of stocking density in triggering aggressive behaviour, especially in species like seabass that are prone to cannibalism under stressful conditions.

Higher densities often exacerbate territorial disputes, which can escalate aggressive behaviours like cannibalism (Khan et al 2021). Survival rates in this study were inversely related to stocking density, with fish at T_1 achieving the highest survival (96.54%) and those at T_3 showing significantly lower survival

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(86.45%). Statistically significant differences could be observed between T_1, T_2 and T_3 ($p < 0.05$).

This inverse relationship between density and survival has been well documented by Ellis et al (2005), who suggested that high stocking densities can lead to deteriorated water quality, increased disease susceptibility and higher mortality rates. Moreover, Sadhu et al (2014) highlighted that stress associated with overcrowding can suppress immune function, thereby, making fish more vulnerable to infections, which may have contributed to the lower survival observed at higher densities in this study.

The findings of this study are consistent with previous research, demonstrating that lower stocking densities in RAS promote superior growth performance, feed utilization and survival, while mitigating aggressive behaviours like cannibalism. These results underscore the importance of optimizing stocking densities in aquaculture systems to enhance fish welfare, minimize stress and maximize production efficiency.

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CONCLUSION

The present study clearly demonstrates that stocking density plays a crucial role in determining the growth performance, behaviour and survival of Asian seabass fry in a recirculating aquaculture system (RAS). Among the tested densities, 100 fish per m^3 (T_1) proved to be the most suitable, resulting in superior growth, better feed utilization, lower cannibalism and higher survival rates. Although water quality parameters remained within acceptable limits across all treatments, increased stocking density led to subtle changes that, along with heightened competition and stress, negatively affected fish performance. Higher densities also intensified cannibalistic behaviour, which significantly reduced survival. Therefore, maintaining an optimal stocking density is essential for improving productivity and ensuring fish welfare in seabass nursery systems.

The findings of this study provide valuable insights for aquaculture practitioners and highlight the importance of density management in RAS-based nursery rearing for sustainable seabass production.

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