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Optimization of feeding frequency of Asian seabass (*Lates calcarifer*) fry reared in net cages under brackishwater environment

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ABSTRACT

An experiment was conducted in brackishwater environment to determine the optimal feeding frequency for growth, effective feed conversion, survival, shooters emergence and size variation in Asian seabass fry reared in the net cages. Four feeding frequencies of one (T1), two (T2), three (T3) and four (T4) times a day were evaluated as treatments in triplicate for a period of 5 weeks. Hatchery produced weaned seabass fry (25.9 ± 0.3 mm/203.8 ± 4.6 mg size) stocked at 120 numbers per cage were fed with a commercial marine fish larval diet containing 55% crude protein at 10% of the biomass daily for the first 3 weeks, followed by 8% for the remaining 2 weeks. Although, the highest growth was recorded in T3, the final length (45.9 ± 0.3 mm) and weight (1203.8 ± 4.6 mg) did not differ significantly ($P>0.05$) from that of T4. Whereas, fish with one or two times feeding exhibited significantly lower growth ($P<0.05$). Daily weight gain, percentage weight gain and specific growth rate were significantly higher in T3 ($P<0.05$), while there was no significant variation ($P>0.05$) between T3 and T4. Significantly higher survival of 75.89 ± 4.17% was recorded in T3 than those of one and two times fed fish ($P<0.05$). The fish in T3 had significantly improved feed conversion ratio ($P<0.05$). No significant differences were recorded among treatments for the cumulative number of shooters separated and coefficient of variation in the harvest weight, which were ranging from 9.67 to 12.00 and 0.113 to 0.124, respectively. This study infers that the Asian seabass fry can achieve maximum growth, survival and better feed conversion when they are fed a given ration with three times feeding daily in brackishwater net cage rearing. The findings also have practical significance towards establishing Asian seabass seed rearing package and will directly benefit the nursery operators.

1. Introduction

The Asian seabass (*Lates calcarifer*) with its wide Indo-Pacific distribution (Greenwood, 1976) has been a high demand food fish and farmed traditionally since the nineteenth in India (Lovatelli, 1990). Successful induced breeding in 1997 (Thirunavukkarasu and Kailasam, 1999) and subsequent development of large scale seed production technology by the Central Institute of Brackishwater Aquaculture have projected its potential as a suitable candidate species for diversified coastal aquaculture. Indeed, owing to its palatability and flesh quality this species has high consumer preference and can be grown in ponds and cages (Barlow et al., 1996; Singh, 2000).

Hatchery produced 25–30 day old seabass fry are reared further for a period of 30–45 day nursery phase, until they attain suitable size for stocking in grow-out culture systems. Generally, seabass nursery is carried out in fertilized brackishwater ponds and in net cages placed inside a pond or in an open coastal area (Kungvankij et al., 1986; Fermin et al., 1996; Fermin and Seronay, 1997; Arasu et al., 2008). Nursery rearing in net cages is advantageous to other methods as it is easily managed and requires less space and capital investment. Seabass fry reared under controlled and confined conditions face competition among individuals for food and space leading to uneven growth causing cannibalism (MacKinnon, 1985; Parazo et al., 1991a). Usually, cannibalism is more frequent in the first 2 months in the presence of shooters or cannibals (Parazo et al., 1991b). During the net cage rearing seabass fry mainly subsist on external feed supply. Considering the above facts for this type of rearing, an appropriate feeding strategy is of paramount importance to produce quality stockable size seeds for grow-out operation. Among the different feed management practices proven to maximise the benefit of feeding, feeding frequency and ration size play an important role in regulating the feed intake, growth and waste outputs of fish (Silva et al., 2007). Optimizing feeding frequency may minimise feed wastage, leading to improvement in culture environment and or reduction in size heterogeneity (Dwyer et al., 2002; Tucker et al., 2006), whereas poorly timed or sporadic feeding frequency may lead to increased hunger, intra-specific aggression and increased rate of cannibalism (Folkvord and Ottera, 1993). All these problems result in decreased

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production efficiency which ultimately increases cost of production (Booth et al., 2008). However, optimum feeding frequency and rate vary depending on the fish species, size and rearing system (Cho et al., 2003). Although several studies have been conducted to determine the optimum feeding frequency for growth, survival, feed intake, body composition etc. in different fish species at their early life stages (Chiu et al., 1987; Abud, 1990; Tsevis et al., 1992; Folkvord and Ottera, 1993; Wang et al., 1998, 2009; Lee et al., 2000; Hossain et al., 2001; Dada et al., 2002; Dwyer et al., 2002; Cho et al., 2003; Schnaittacher et al., 2005; Tucker et al., 2006; Biswas et al., 2006a,b; Silva et al., 2007; Booth et al., 2008), lack of information exists in this regard for Asian seabass fry reared in net cages. However, in a study on this species, Salama (2008) reported that feeding two times daily resulted in better growth and feed utilization for juveniles (>40 g) reared under hypersaline seawater of the Red Sea. Therefore, the present experiment in net cages aimed at optimization of feeding frequency for growth, feed conversion, survival, shooter emergence and size variation in Asian seabass fry.

2. Materials and methods

2.1. Experimental site and arrangement

This experiment was carried out in the brackishwater tide-fed farm of the Kakdwip Research Centre of Central Institute of Brackishwater Aquaculture (CIBA), Kakdwip (Lat. 21°51′15.01″–21°51′30.77″ N, Long. 88°10′58.44″–88°11′12.09″E), South 24 Parganas, West Bengal, India for a period of 5 weeks. One pond of 600 m^2 area was prepared ready by application of lime stone powder at 250 kg ha^{-1} 1 week prior to start of the experiment. Twelve nylon net (mesh size = 0.71 × 0.45 mm) cages of size 1 × 1 × 1.2 m (length × breadth × height) were fixed in the pond at the both sides of a catwalk kept 0.2 m height above the water surface.

2.2. Experimental procedure and management

Seabass fry produced by induced breeding at the CIBA hatchery were gradually weaned to a commercial marine fish larval diet (INVE Ltd., Thailand; composition: 55% crude protein, 9% lipid, 1.9% fibre and 8% moisture; particle size 500 µm). Weaned 25-day old fry were brought to the experimental site, stocked in net cages and fed a fixed ration (10% biomass d^{-1}) of the same diet at two meals per day for 1 week in order to acclimatize to the pond based cage rearing conditions. After acclimatization, all the fry were collected and uniform size healthy ones were segregated. The initial mean length and weight were computed taking 50 fry samples thrice from a common population. Total length was measured to the nearest 0.1 mm. Individual body weight was determined to the nearest 0.1 mg using a digital analytical balance. Selected fry (25.9 ± 0.3 mm/203.8 ± 4.6 mg) were then stocked in the twelve net cages distributing 120 numbers uniformly in each cage. Before being stocked into the cages, the fry were subjected to a short dip in a mild $KMnO_4$ solution as a prophylactic measure.

Feed in each net cage was provided at 10% of biomass per day (Lee et al., 1996) for the first 3 weeks and 8% for the remaining 2 weeks. The fry were fed with the previously mentioned diet with particle size gradually increased from 500 to 800 µm over the course of experiment to fit changes in fish mouth size. Feed ration was adjusted weekly after estimating the biomass through intermediate samplings and counting the numbers survived. Keeping the daily rations identical, differential feeding frequencies were chosen as the variable. Feeding frequencies of one, two, three and four times per day were defined as the four treatments with triplicate net cages for each treatment selected at random. The feeding frequency and schedule followed in different treatments are presented in Table 1. Pond water samples were collected between 09:00 and 10:00 h at weekly intervals to measure important parameters. Temperature, dissolved oxygen, pH and total alkalinity were recorded following standard methods (APHA, 1998) and salinity was assessed using a refractometer (ATAGO, Japan).

Table 1
Daily feeding frequency and time of feeding in different treatments.

Treatment	Frequency	Feeding time
T1	One	07:00 h
T2	Two	07:00 and 16:00 h
T3	Three	07:00, 11:30 and 16:00 h
T4	Four	07:00, 10:00, 13:00 and 16:00 h

2.3. Fish sampling and separation of shooters

Periodic samplings of fish were performed to assess the growth performance and separate the shooters weekly. The weekly mean length and weight increments were obtained from three random samples of 10 individual fish from each net cage. At first, the average body weight of the growing fishes was calculated with inclusion of shooters in the samples. The shooters which had a minimum size difference of approximately 33% from the rest of the stock (Parazo et al., 1991a,b) were then removed without replacement. Lastly, average body weight of fish excluding the shooters in each cage was computed from the sampling data and weekly feed adjustment was made accordingly. Further, the cumulative number of shooters separated was calculated by aggregating the weekly values and the value at harvest, whereas, the mean final length, weight and survival of fish were recorded at the time of harvest. Weekly separated shooters were stored apart and reared in a net cage till the end of the experiment and they were fed with the same diet. Final individual weight of shooters from the isolated net cage was recorded during harvest and mean weight was calculated. Cages were replaced with new ones immediately after sampling. Used cages were brushed thoroughly to remove algae and silt that clogged the mesh and were then sun-dried.

2.4. Fish performance evaluation

Fish performances in net cages were evaluated in terms of final length (mm), weight (mg), daily weight gain (DWG, mg d^{-1}), percentage weight gain (PWG, %), specific growth rate (SGR, % d^{-1}), survival (%), feed conversion ratio (FCR), cumulative number of shooters separated and coefficient of variation. Growth, growth related indices (DWG, PWG and SGR), FCR and survival were calculated without considering the discarded shooters. Coefficient of variation was computed considering the shooters recorded during harvest. These performance indices for each net cage were calculated as follows.

- DWG = (mean final weight − mean initial weight) / rearing duration in days
- PWG = [(mean final weight − mean initial weight) / mean initial weight] × 100
- SGR = [(ln final weight − ln initial weight) / rearing duration in days] × 100
- FCR = total feed intake / total biomass gain
- Survival = (number of fish harvested / number of fish stocked) × 100
- Coefficient of $variation_{harvest\ weight}$ (CV_{hw}) = mean standard deviation of final weight / mean final weight of fish

Note: for FCR calculation, because of practical difficulty for collection of uneaten feed for this kind of field study, it was assumed that the total amount of feed provided in each net cage was consumed by fish.

Table 2
Variations in water quality parameters of the experimental site.

Parameter	0 day	7th day	14th day	21st day	28th day	36th day
Temperature (°C)	31	30	31	29	31	26
pH	7.97	7.95	7.90	7.70	7.75	8.07
Dissolved oxygen ($mg\ l^{-1}$)	7.2	7.0	8.0	8.0	7.6	8.0
Total alkalinity ($mg\ CaCO_3 l^{-1}$)	180	184	160	172	176	160
Salinity ($g\ l^{-1}$)	4.1	3.7	3.2	3.5	3.6	3.6

2.5. Statistical analysis

Differences in final length, final weight, DWG, PWG, SGR, FCR, survival, number of shooters separated and CV_{hw} among fish fed at different frequencies were determined by analysis of variance with the General Linear Model procedure using SPSS for Windows v.17.0 programme (SPSS Inc., Chicago, IL, USA). Duncan's Multiple Range Test (Duncan, 1955) was used for comparison of treatments. All data are expressed as mean ± standard error (S.E.).

3. Results

General water quality in the experimental pond did not exhibit any marked variations (Table 2). Salinity of the experimental site varied from 3.2 to 4.1 $g l^{-1}$ throughout the rearing duration. Fry were healthy and no infection or disease was encountered during sampling. All indices and data recorded and calculated at the end of experiment according to feeding frequency are presented in Table 3.

3.1. Growth, DWG, PWG and SGR

The feeding frequencies tested for this study affected the growth in terms of final length and weight (Fig. 1). The highest growth was recorded in T3, while the final length (45.9 ± 0.3 mm) and weight (1203.8 ± 4.6 mg) did not differ significantly ($P>0.05$) from that of T4. However, fish in T3 and T4 attained significantly higher length and weight ($P<0.05$) than those of T1 and T2. Daily weight gain (DWG), percentage weight gain (PWG) and specific growth rate (SGR) values were higher in T3 followed by T4, T1 and T2. Although, DWG (28.6 ± 0.3 $mg\ d^{-1}$), PWG (490.61 ± 5.39%) and SGR (5.07 ± 0.03% d^{-1}) in T3 were not significantly different from that of T4 ($P>0.05$), they were significantly higher than those of T1 and T2, whereas, T4 and T1 were not different ($P>0.05$) for these indices. T2 recorded the lowest values for DWG (26.8 ± 0.2 $mg\ d^{-1}$), PWG (460.75 ± 3.62%) and SGR (4.93 ± 0.02% d^{-1}), which did not differ significantly ($P>0.05$) from T1.

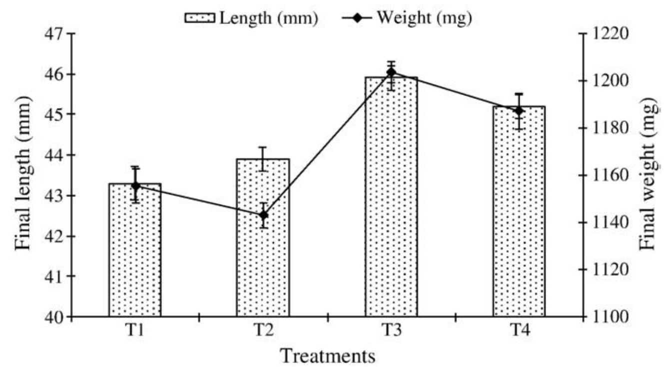

Fig. 1. Comparison of final growth (length and weight) of seabass under different treatments.

3.2. Feed conversion ratio

In this study, FCR was significantly affected by the feeding frequency. Fish in the T3 showed a significantly better FCR of 2.23 ± 0.12 ($P<0.05$) than the groups fed one, two and four times daily with values of 2.88 ± 0.20, 3.04 ± 0.19 and 2.85 ± 0.04, respectively. FCR in T1, T2 and T4 did not differ significantly ($P>0.05$).

3.3. Survival

The highest survival of 75.89 ± 4.17% was recorded in T3, which was not different ($P>0.05$) from that of T4 (69.72 ± 2.37%). However, survival in T3 was significantly higher ($P<0.05$) than the values obtained for T1 (56.39 ± 7.01%) and T2 (58.61 ± 5.28%).

3.4. Shooters separated

The mean cumulative number of shooters separated was highest in T1 (12.00 ± 0.58) followed by T2 (11.33 ± 0.33), T3 (10.67 ± 0.88) and T4 (9.67 ± 1.45) with no significant difference ($P>0.05$). With the progress of rearing period the number of shooters sorted out from each treatment revealed almost a decreasing trend (Fig. 2). Separated shooters reared apart attained a final mean weight of 8772.7 ± 1065.1 mg.

3.5. Size variation

Feeding frequency did not significantly affect size variation of harvested fish ($P>0.05$). The mean CV_{hw} was minimum in T3 (0.113 ± 0.008) and maximum in T1 (0.124 ± 0.008).

Table 3
Comparison among treatments for final length, final weight, DWG, PWG, SGR, survival, FCR, number of shooters separated and CV_{hw} of Asian seabass, *Lates calcarifer* under different feeding frequencies.

Parameters	Treatments			
	T1	T2	T3	T4
Final length (mm)	43.3 ± 0.4^{b}	43.9 ± 0.3^{b}	45.9 ± 0.3^{a}	45.2 ± 0.3^{a}
Final weight (mg)	1155.5 ± 7.3^{b}	1143.0 ± 5.3^{b}	1203.8 ± 4.6^{a}	1187.2 ± 7.5^{a}
DWG ($mg\ d^{-1}$)	27.2 ± 0.4^{bc}	26.8 ± 0.2^{c}	28.6 ± 0.3^{a}	28.1 ± 0.2^{ab}
PWG (%)	466.91 ± 6.94^{bc}	460.75 ± 3.62^{c}	490.61 ± 5.39^{a}	482.46 ± 4.14^{ab}
SGR ($\%\ d^{-1}$)	4.96 ± 0.03^{bc}	4.93 ± 0.02^{c}	5.07 ± 0.03^{a}	5.04 ± 0.01^{ab}
FCR	2.88 ± 0.20^{a}	3.04 ± 0.19^{a}	2.23 ± 0.12^{b}	2.85 ± 0.04^{a}
Survival (%)	56.39 ± 7.01^{b}	58.61 ± 5.28^{b}	75.89 ± 4.17^{a}	69.72 ± 2.37^{ab}
Shooters separated (nos.)	12.00 ± 0.58^{a}	11.33 ± 0.33^{a}	10.67 ± 0.88^{a}	9.67 ± 1.45^{a}
CV_{hw}	0.124 ± 0.008^{a}	0.122 ± 0.010^{a}	0.113 ± 0.008^{a}	0.114 ± 0.008^{a}

Means with different superscripts differ significantly in a row ($P<0.05$); values are means ± S.E. of three replicates.

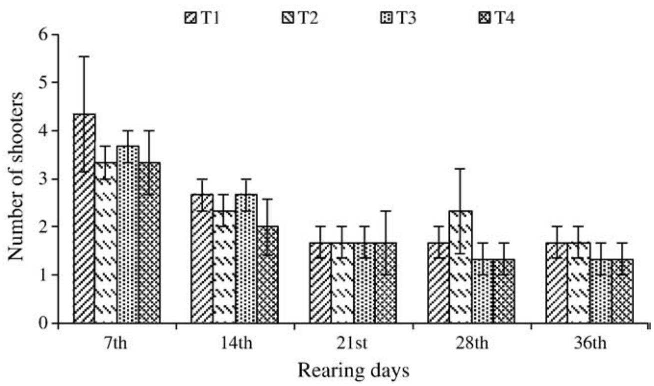

Fig. 2. Weekly emergence of shooters in treatments with different feeding frequencies.

4. Discussion

General water quality parameters of the experimental site were within the optimum ranges for seabass seed rearing (Kailasam et al., 2006). However, salinity was in low range (3.2 to 4.1 $g\,l^{-1}$) which is mainly due to seasonal salinity variation of the tidal water in this region (Saha et al., 2001).

In the present experiment, feeding frequency had significant effects on the growth, survival and FCR of Asian seabass. The observations are consistent with the results obtained by Wang et al. (1998), Lee et al. (2000), Dwyer et al. (2002), Harpaz et al. (2005), Kikuchi et al. (2006), Booth et al. (2008) and Wang et al. (2009) who reported that feeding frequency very often affects the weight gain, feed intake, food consumption and feeding behaviour of cultured fish.

This study revealed that growth in terms of final length and weight and other weight related indices, viz. DWG, PWG and SGR in seabass fry could be improved when they were reared with feeding provided for three or four times compared to one and two times daily. Although these indices did not vary significantly ($P>0.05$) between fish fed three times daily and four times daily, three feedings daily produced larger size fish. These growth performance results are higher than the observations reported in seabass fry with mean length of around 41 mm in 45 day rearing (Kailasam et al., 2006) and 42.1 mm (1311.8 mg) in 42 day rearing in floating net cages (Fermin et al., 1996). However, in another experiment performed in net cages, Biswas et al. (2008) demonstrated slightly higher growth of 46.9 mm (1560 mg) when the fry were reared with formulated feed for 30 days at a density of 120 numbers m^{-2}. Fish show different growth responses to feeding frequency and growth increases with increasing feeding frequency up to a certain level as was observed in common carp fry (Charles et al., 1984), red-spotted grouper (Kayano et al., 1993), rainbow trout (Ruohonen et al., 1998), yellowtail flounder (Dwyer et al., 2002), Australian snapper (Tucker et al., 2006), tiger puffer (Kikuchi et al., 2006) and pikeperch (Wang et al., 2009). On the contrary, daily feeding frequency did not affect growth of larvae and juveniles of African catfish (Kaiser et al., 1995), juvenile Korean rockfish (Lee et al., 2000), fry of rohu and mrigal (Biswas et al., 2006a). As growth was not significantly enhanced by increasing the number of meals from three to four, feeding three times a day seems to be sufficient for maximal growth in Asian seabass fry under net cage rearing conditions. Though the reason for this observation is not clear, it may be attributed to the fact that when the interval between meals is short, the food passes through the digestive tract more quickly, resulting in less effective digestion (Liu and Liao, 1999) and assimilation. In addition, repeated feeding throughout long periods of the day would increase swimming activity of fish, leading to energy expenditure and lower growth rate (Johansen and Jobling, 1998).

The effect of feeding frequency on growth performance changes with the size of fish. Generally, fry are fed smaller meals more frequently, while feeding once or twice a day is sufficient for broodstock and larger fish. Usually feed amount required for optimal growth diminishes as fish grows. In this line, Pullin and Lowe-McConnell (1982) suggested feeding tilapia, *Oreochromis niloticus* 12 times daily when their weight is 20 g but only twice daily when they weigh 200 g. Rainbow trout (0.3 g) grew best when fed eight times daily, but 15 g fish attained maximum growth when fed 3 meals per day (Piper, 1982). In another instance, Kikuchi et al. (2006) recommended feeding three times a day for fish smaller than 100 g and once a day for larger fish to be adequate for production of tiger puffer. The present finding suggests three times feeding daily for *L. calcarifer* fry, whereas, during rearing in hypersaline water (salinity 43–44 $g\,l^{-1}$) Salama (2008) demonstrated that feeding two times per day produced significantly higher growth in juveniles (>40 g) of the same species.

Significantly better FCR value was obtained by the fish group fed three times daily than the other three groups provided with one, two and four times daily ($P<0.05$). Similarly, Charles et al. (1984) and Wang et al. (2009) found that feed efficiency was highest in common carp and pikeperch, respectively when they were fed three times a day. Feeding fish at greater frequencies than they can accommodate could lead to inefficient use of feed and poor FCRs (Schnaittacher et al., 2005). Likewise, the evidence of significantly poorer FCR in fish group fed four times daily was due to increased 'gastrointestinal overload' where intake of the next meal occurred before the previous bolus had been subjected to adequate gastric attack (Booth et al., 2008). When this occurred, the existing chyme entered the anterior intestine only partially digested (Riche et al., 2004). Reducing the feeding frequency to one or two times per day, significantly decreased growth and increased FCR in seabass fry. One possible reason for this is that the less frequently fed fishes could not meet up the nutrient and energy requirements for their maintenance and somatic development.

Feeding frequency affected the survival in seabass fry with fish fed three meals daily exhibiting the highest survival. Significantly lower survival was found in fish groups fed at one and two feeding frequencies. Present observation of feeding frequency influencing survival was in accordance with the conclusions of Chua and Teng (1978), Carlos (1988) and Goldan et al. (1997) for the fish species of *Epinephelus tauvina*, *Aristichthys nobilis* and *Sparus aurata*, respectively.

Seabass fry is highly carnivorous and voracious feeder and occurrence of shooters during larval rearing drastically reduces the survival through cannibalism (Parazo et al., 1991a and Kailasam et al., 2002). So, it is necessary to sort out and size-grade them regularly to minimise competition for space and food, thus reducing cannibalism. In this study, non-significant reduction of cumulative number of shooters sorted out on weekly basis occurred with the increasing feeding frequency. Also, as the experiment progressed the number of shooter emergence decreased gradually almost for all the fish groups reared with four different feeding frequencies. It indicates that the shooters occur more in numbers in smaller individuals during the early stage of life (Rutledge, 1991; Kailasam et al., 2002; Biswas et al., 2008). The separated shooters reared apart attained 6.29 times higher growth than the largest grown fish group fed three times daily. It is a clear sign of good potential of these shooters to grow faster than the smaller individuals and when they are farmed separately would reach marketable size quickly.

It is important to minimise size heterogeneity in aquaculture practice in order to maintain uniform size fish. Additionally, a uniform size distribution limits the formation of size hierarchies and social dominance which may lead to aggression (Thomassen and Fjaera, 1996; Greaves and Tuene, 2001) and cannibalism. It has been suggested that more frequent feedings, at a given ration level, may produce fish of more uniform sizes (Wang et al., 1998), because smaller fish might get more chances to obtain food (Schnaittacher et al., 2005). Similar to the present observation, low CV values (12.2–13.4%) were reported for European sea bass, *Dicentrarchus labrax* reared in outdoor tanks for around 78 days with initial average body weight of 64.2 g (Azzaydi et al., 1998). However, we observed that there was no significant size heterogeneity among fish fed one to four meals a day. As the relative importance of size variation seems to be greater for small fish than larger ones and a prolonged period with large size differences substantially reduces survival (Folkvord and Ottera, 1993), conductance of weekly size-grading and sorting out of shooters as recommended by Parazo et al. (1991a), Fermin et al. (1996) and Kailasam et al. (2002) for this carnivorous fish, *L. calcarifer* certainly reduced the size variability in a greater extent among the fishes fed at different feeding frequencies.

5. Conclusion

Success of a commercial aquaculture operation largely depends on the growth and survival of the fish under culture. As feed is the single most significant cost involved, it is emphasised to carry out farming with its maximum conversion into fish growth in a cost-effective management approach. Considering these facts, this study suggests that in brackishwater net cage rearing, Asian seabass fry can achieve maximum growth, survival and better feed conversion when they are fed a commercial diet containing approximately 55% crude protein, at a given ration with three times feeding daily. Other management steps like regular size-grading and separation of shooters would assist to raise uniform size fish and increase survival. Additional information for this species is anticipated regarding the role of gastrointestinal activities influencing the feeding behaviour and feed utilization at different feeding frequencies at the initial life stage. The findings of the current study have practical significance as a major step for establishing Asian seabass seed rearing practice package and will directly benefit the nursery operators.

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