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Optimization of stocking density of seabass (*Lates calcalifer*) in brackish- and freshwater earthen ponds under monoculture in South-west coastal zone of Bangladesh

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Abstract. The Asian seabass (*Lates calcarifer*) is a fast-growing, euryhaline fish capable of withstanding wide environmental fluctuations and fetch higher market price due to its good taste. For semi-intensive and intensive aquaculture, it is important to optimize stocking density. The present study was undertaken to compare and standardize production and stocking density of seabass in earthen ponds. Seabass fingerlings were collected from natural sources and stocked both in coastal brackishwater and freshwater ponds with the stocking density of 20, 25 and 30 individual/decimal in T1, T2 and T3, respectively. The seabass feed prepared with 40% crude protein, 8% crude lipid, 10.00% ash and 3.00% crude fibre was applied as supplementary feed. Water quality parameters were not significantly different among the treatments. In brakishwater ponds, average survival rates (%) were 59.31±3.08%, 53.75±2.78% and 39.99±2.09% in T1, T2 and T3 respectively while in freshwater ponds the survival rates were 58.81±3.90%, 51.82±5.84% and 39.59±2.06% in T1, T2 and T3 respectively. The specific growth rates (SGR) of seabass in brackishwater was somewhat higher in T2 without significant difference among the treatments. The SGR of seabass in freshwater pond was significantly higher in T2 and T3 than that of T1. But the highest SGR was recorded in T2 where stocking density was 25 individual/decimal. Average net yields in treatments T1, T2 and T3 under brackishwater were estimated as 1043.06±34.13 kg/ha, 1255.55±58.63 kg/ha and 1115.18±70.29 kg/ha respectively. Average net yields in treatments T1, T2 and T3 under freshwater were 946.96±93.73kg/ha, 1186.79±113.46kg/ha and 1069.86±33.04kg/ha respectively. The highest net yields in T2 were 1255.55±58.63 kg/ha and 1186.79±113.46 kg/ha in brackish water and freshwater environment, respectively with significant difference among the treatments. According to the findings of the present study, it may be concluded that the culture of seabass at stocking density of 25 individual/decimal in earthen pond of brackishwater environment with artificial feed has profound impacts on SGR which ultimately increases the yield of seabass.

Keywords: Asian seabass, Stocking density, Water quality parameters

Introduction

The Asian seabass or barramundi perch, *Lates calcarifer* is a diadromous (Kungvankij *et al.* 1986) euryhaline fish of Indian sub-continent belongs to Centropomidae family. It is locally called as "Coral" or "Vetki" in Bangladesh. The seabass is one of the most commercially and recreational significant species for both aquaculture and capture fisheries. It grows to a reasonably large size with delicate flavored flesh and fetches a high (Das 2000, Luna 2008). The Asian seabass is a fast-growing, euryhaline fish capable of withstanding wide environmental fluctuations (Thirunavukkarasu *et al.* 2004, Singh 2000). Having the characteristics of catadromous pattern of life cycle, it's population occupies a wide range of habitats starting from freshwater rivers, estuaries and inshore coastal waters. Therefore, it can be cultured in a variety of culture systems using marine water, brackish water and freshwater (Harpaza *et al.* 2005,

FAO 2006). It is unfortunate that the seabass resource has become vulnerable in marine environment due to over exploitation (Cheung *et al*. 2005). To overcome this problem, many south-east Asian countries gave concerted efforts over the last couple of decades and developed seed production and commercial aquaculture techniques of seabass (Almendras *et al*. 1988, Rimmer *et al*. 1994, Thirunavukkarasu *et al*. 2001). Farming based on supplementary feed was conducted by using different types of feeds like trash fish, crustacean pellets, formulated feed, and live food (Biswas *et al*. 2010, Boonyaratpalin *et al*. 1998, Bermudes *et al*. 2010). Standardization of optimum stocking density for pond culture of seabass and standardization of optimum forage fish quantity for culture of seabass were evaluated in India (Singh *et al*. 2012). Effects of stocking density on the survival and growth of Asian seabass cultured in open sea floating cages were investigated in India (Sadhu *et al*. 2015). Some interventions were made by Kamruzzaman *et al*. (2013), Monwar *et al*. (2013), James and Marichamy (1986) for increasing seabass aquaculture in earthen pond.

In Bangladesh, seabass is found to be inhabited the Bay of Bengal, estuaries of Barishal, Patuakhali and Khulna of the south-western coastal region (Rahman 1989). A few researches have been done so far on seabass culture in Bangladesh. Most of the experiments were focused on culture of seabass with tilapia where tilapia fries were used as seabass food. But for commercial and semi-intensive/intensive aquaculture, it is important to culture seabass with artificial feed and to optimize stocking density. Another important issue is to culture the seabass both in the brackish and freshwater ponds with artificial feed that would be helpful for ensuring higher growth of marine seabass both in brackish and freshwater environment. Considering the fact, the present experiment was undertaken to compare the production of seabass in brackish and freshwater earthen pond and to determine the stocking density of seabass in earthen pond.

Materials and Methods

Collection and stocking of experimental fish in brakishwater and freshwater ponds: The experiments were conducted in freshwater (0 ppt) and brakish water (5-10 ppt) ponds located in Kalapara, Dumki, Bauphal Upazila of Patuakhali District from July 2017 to September 2018. Nine (9) freshwater ponds and 9 brackish water ponds were prepared as per standard procedure as grow-out ponds with average area and depth of 15 decimal and 1.50 meter respectively. Two (2) nursery ponds (15 decimal) were also prepared as per standard pond preparation protocol for nursing the fish fries. Seabass seeds were collected from natural sources of Andharmanik River, Kalapara and stocked in nursery ponds. The mean initial weight of seabass ranged from 59.00 to 71.00 g at the time of stocking. The stocking density in three different treatments are shown in Table I. Natural food productions in the ponds were enhanced by applying fertilizers (both organic and inorganic). The fish were fed with artificial feed with 40% protein.

Table I. Design on stocking density of seabass in freshwater and brakishwater ponds

Environment	Brackishwater			Freshwater		
Treatments	T1	T2	T3	T1	T2	T3
Replication	3	3	3	3	3	3
Stocking (Fish/Dec.)	20	25	30	20	25	30
Feed	Std. Regime: Artificial & natural Feed			Std. Regime: Artificial & natural Feed		
Salinity	5-10 ppt			0 ppt		

***Proximate composition of fish feed*:** Proximate composition of feed was chemically analyzed according to the standard methods of Association of Official Analytical Chemists (AOAC) (2000) for moisture, crude protein, crude lipid, crude fibre and ash. Proximate composition analysis of seabass feed (Quality feed Ltd) was done and their composition in percentage are shown in Table II.

Table II. Proximate composition of seabass feed (% dry basis)

Parameters	Amount (%)
Moisture	11.00
Crude protein	40.00
Crude lipid	8.00
Ash	10.00
Crude fibre	3.00
Carbohydrate	28.00

***Measurement of water quality parameter*:** Water quality parameters temperature, dissolved oxygen (DO), pH and salinity were measured with respective meters. Nitrate-nitrogen, ammonia-nitrogen, phosphate-phosphorus and alkalinity of experimental ponds water were measured with HACH spectrophotometer, model: DR 6000.

Estimation of growth, yield and survival of fish: Fish were harvested at the end of the experiment in July 2018. During stocking and harvest, all fish were weighed and counted pond wise. The following parameters were used to determine fish yield:

Average final weight gain = Average final weight – Average initial weight

$$\text{Specific growth rate (SGR, \% bwd-1)} = \frac{\text{Ln final weight - Ln initial weight}}{\text{Culture Period}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed fed (dry weight)}}{\text{Live weight gain}}$$

Survival rate (%) = (No. of fish caught/ No. of fish released) ×100
Gross yield = No. of fish caught × Average final weight
Net yield = No. of fish caught × Average weight gained

***Statistical analysis*:** For the statistical analysis of the data, Duncan's Multiple Range Test (DMRT) was done by using the SPSS (Statistical Package for Social Science) version- 20.0. Significance was assigned at the 5% level.

Results and Discussion

***Water quality parameters in brackish water and freshwater ponds*:** The mean values of water temperature in the fish ponds fluctuated between 29.50 and 30.83°C among the different treatments of the present study (Table III) which are almost similar to the values of 26-31°C and 29-32 °C in seabass ponds reported by Biswas *et al*. (2010) and Monwar *et al*. (2013).

Dissolved oxygen (DO) values ranged from 4.75-5.83 mg/l (Table III). The present study reported lower DO values than the findings (7.2-8.0mg/l) of Biswas *et al*. (2010). Monwar *et al*. (2013) also reported wide ranges of DO values (3.9-8.9 mg/l) in seabass ponds which are higher than the values obtained in the present study. The present study reported pH values from 7.25 to 8.00 among the different treatments, which are the suitable condition for fish culture (Table III). A similar result (7.70-8.07) was reported by Biswas *et al*. (2010). The salinity of brackish water ponds was ranged from 6.07-6.39 ppt whereas freshwater salinity was 0 (zero) in all treatment groups (Table III). The range of salinity values recorded by Biswas *et al*. (2010) in seabass ponds was 3.2-4.1 ppt which is lower than the values obtained in the present study. Monwar *et al*. (2013) reported salinity ranged were 0 to 6 ppt in seabass ponds which are lower than the values obtained in the present study.

Table III. Water quality in brackish- and freshwater earthen ponds during study

Parameters	Brackish water			Freshwater		
	T1	T2	T3	T1	T2	T3
Water temp. (°C)	30.25±1.2	30.50±1.3	30.25±1.2	29.67±1.6	30.83±1.6	29.50±0.5
DO (mg/l)	4.75±0.9	5.50±0.6	5.50±0.6	5.83±0.4	5.00±0.9	5.33±0.8
pH	7.75 ± 0.5^{ab}	8.00 ± 0.0^{b}	7.25 ± 0.5^{a}	7.68±0.2	7.60±0.3	7.65±0.3
Nitrate-N (mg/l)	0.25±0.50	0.10±0.05	0.09±0.04	0.05±0.02	0.08±0.0	0.33±0.52
Phosphate-P (mg/l)	1.25±0.50	1±0.00	1.25±0.50	0.69±0.4	0.14±0.1	0.14±0.1
Ammonia-N (mg/l)	0.20± 0.1	0.14±0	0.16± 0.05	0.17± 0.1	0.16±0.1	0.25±0.2
Alkalinity (mg/l)	195.5±41	174.4±4	188.8±61.8	165.44±4	173.44±31	189.33±30
Salinity (ppt)	6.10± 1	6.07± 0.78	6.39±0.9	0	0	0

Mean values with different superscript in one row are statistically significant at $p < 0.05$.

Nitrate-nitrogen (NO_3-N) is a very essential chemical parameter to maintain good productivity of the water body. We observed 0.05-0.33 mg/l NO_3-N (Table III), which was almost similar to the findings of Ali (1992). Azim *et al*. (1995) stated that near about 0.5 mg/l nitrate-nitrogen was suitable for fish culture. According to their statements, the values of the nitrate-nitrogen recorded in the present study were suitable for fish culture. The concentrations of phosphate-phosphorus (PO_4-P) ranged between 0.14-1.25 mg/l which is almost similar to the range (0.51-1.23 mg/l) and (0.32-1.00) reported by Kunda (2003). The ranges of ammonia-nitrogen (mg/l) obtained were 0.14-0.25 mg/l (Table III). Concentration of ammonia showed an increasing trend as the days of culture increased, probably due to higher metabolic deposition and organic load. The range of ammonia-nitrogen values recorded by Mohanty *et al*. (2004) in rice fields were 0.01-0.31 mg/l which are almost similar to the values obtained in the present study. The ranges of alkalinity (mg/l) obtained were 165.44-195.41 mg/l in the present study (Table III). The range of alkalinity values recorded by Biswas *et al*. (2010) in seabass ponds were 160-184 mg/l which are almost similar to the values obtained in the present study. Monwar *et al*. (2013) reported alkalinity ranged were 24 to 78 mg/l in seabass ponds which are invariably lower than the values obtained in the present study.

Growth performance of seabass in brackish and freshwater under different treatments

***Final weight (g)*:** Mean final weights of seabass in brakishwater ponds were 417.07±17.00g, 440.07±12.53g and 432.20±3.24g in T1, T2 and T3 respectively (Table IV). Higher mean

final weight of seabass was obtained 440.07±12.53g in T2 compared to other treatments without significant difference among treatments. Whereas, mean final weight of seabass in freshwater ponds were 391.60±16.93g, 436.80±30.57g and 417.53±16.64g in T1, T2 and T3 respectively, with higher final weight found 417.53±16.64g in T2 compared to other treatments without significant difference among treatments. All the treatments in brackishwater contributed higher final weight of seabass compared to those of freshwater ponds.

Table IV. Growth performance of seabass observed in different treatments

Parameters	Brackish water			Freshwater		
	T1	T2	T3	T1	T2	T3
Stocking (/dec)	20	25	30	20	25	30
Initial weight (g)	60.67±2.1	61.67±5.8	56.00±7.5	66.00 ± 3.6^{b}	65.00 ± 10^{b}	52.33 ± 2.5^{a}
Final weight (g)	417.07±17	440.07±13	432.20±3.2	391.60±16.9	436.80±31	417.53±16.6
Survival rate (%)	59.31 ± 3.1^{c}	53.75 ± 2.8^{b}	39.99 ± 2.1^{a}	58.81 ± 3.9^{b}	51.82 ± 5.8^{b}	39.59 ± 2.1^{a}
SGR (%/day)	1.08±0.1	1.15±0.0	1.14±0.0	0.99 ± 0.1^{a}	1.13 ± 0.0^{b}	1.11 ± 0.1^{b}
FCR	2.68±0.6	2.85±0.6	2.78±0.8	2.71±0.4	2.78±0.4	2.98±0.3
Gross production (kg/ha)	1221 ± 43^{a}	1461 ± 86^{b}	1280 ± 58^{a}	1139 ± 116^{a}	1392.46 ± 114^{b}	1223.26 ± 30^{ab}
Net production (kg/ha)	1043 ± 34^{a}	1256 ± 59^{b}	1115 ± 70^{a}	946.96 ± 94^{a}	1186.79 ± 113^{b}	1069.86 ± 33^{ab}

Mean values with different superscripts in row are statistically significant by at <0.05.

***Survival rate (%)*:** In brakishwater ponds, average survival rates (%) of seabass were 59.31±3.08%, 53.75±2.78% and 39.99±2.09% in T1, T2 and T3 respectively with significantly higher survival rate in T1 (59.31%) and lower in T3 (39.99%). The survival rate (%) of seabass in freshwater ponds were 58.81±3.90%, 51.82±5.84% and 39.59±2.06% in T1, T2 and T3 respectively with significantly higher survival rate obtained in T1 (58.81%) and lower in T3 (39.59%) (Table IV).

***Specific Growth Rate (SGR) (%/day)*:** Average Specific Growth Rates (SGR) of seabass in brakishwater ponds were 1.08±0.05 %/day, 1.15±0.03 %/day and 1.14±0.02 %/day in T1, T2 and T3 respectively. In case of freshwater pond, average SGR of seabass were found 0.99±0.04 %/day, 1.13±0.07 %/day and 1.11±0.05 %/day in T1, T2 and T3 respectively (Table IV). The SGR of seabass was significantly higher in T2 and T3 than the treatment T1. But the highest SGR was recorded in T2 where stocking density of seabass was 25 individual/decimal. The treatments carried out in brakishwater ponds gained higher SGR than those of freshwater ponds.

***Feed Conversion Ratio (FCR)*:** In brakishwater conditions, average Feed Conversion Ratios (FCR) of seabass were calculated as 2.68±0.61, 2.85±0.59 and 2.78±0.85 in T1, T2 and T3 respectively. However, no significant difference of FCR was found among the treatments. In freshwater ponds, average FCR of seabass were 2.71±0.41, 2.78±0.43 and 2.98±0.32 in T1, T2 and T3 respectively. The treatments conducted in freshwater gained somewhat higher FCR than the treatments conducted in brackish water (Table IV).

***Yield of Fish*:** In brakishwater ponds, average gross yields in treatments T1, T2 and T3 were obtained 1220.73±42.47 kg/ha, 1460.78±86.46 kg/ha and 1280.43±57.63 kg/ha respectively.

Average net yields in treatments T1, T2 and T3 were estimated as 1043.06±34.13 kg/ha, 1255.55±58.63 kg/ha and 1115.18±70.29 kg/ha respectively. The highest yield of seabass was recorded in T2 compared to those of other treatments which might be associated with highest metabolic growth rate of fish obtained in this treatment. In freshwater ponds, average gross yields in treatments T1, T2 and T3 were 1139.02±115.55kg/ha, 1392.46±114.00kg/ha and 1223.26±30.21kg/ha respectively. Average net yields in treatments T1, T2 and T3 were 946.96±93.73kg/ha, 1186.79±113.46kg/ha and 1069.86±33.04kg/ha respectively. The highest yield of seabass was recorded in T2 which might be associated with highest specific growth rate of fish obtained in this treatment. Seabass monoculture contributed higher yield in brakishwater ponds compared to those of freshwater ponds (Table IV). The observed moisture, crude protein, crude lipid, ash and crude fibre level were 11.00, 40.00, 8.00, 10.00, 3.00% respectively. Biswas *et al*. (2010) applied seabass feed with 55% crude protein, 9% lipid, 1.9% fibre and 8% moisture which performed better than the feed applied in the present study. Harpaz *et al*. (2005) demonstrated that seabass feed in the form of sinking extruded pellets—3 mm in diameter, containing 48% protein, 12% fat, 1.6% fiber results higher growth performance compared to the present study.

The final weight ranged from 391.60-440.07g among the different treatments of the present study which was lower than the final weight range 200-1390g of Seabass as reported by Monwar *et al*. (2013). Daet (2019) recorded final weight of seabass in hapa-in-pond with initial weight of 139.59-152.34 g after 90 days rearing which was higher than the present study compared to the rearing period. The survival rate of seabass (39.59-59.31%) was found quite lower compared to the investigation carried out by Monwar *et al*. (2013). Daet (2019) recorded 48-90% survival rate of seabass in hapa-in-pond with different stocking density which were higher than the present study (59.7-78.3%). However, Imelda-Joseph (2010) observed poor survival rate at the end of his study mainly due to cannibalism. Other factors which could possibly contribute to low survival rate of seabass include space, presence of natural food to sustain the stock, feeding rate and food utilization and the feeding. Harpaz *et al*. (2005) reported the survival rate of seabass was 90-97% which was higher than the values in the present study. The SGR range obtained 0.99-1.15 %/day among different treatments was lower than the SGR 1.51-1.65 %/day of seabass reported by Daet (2019) who conducted research on seabass in hapa-in-pond with different stocking density. Harpaz *et al*. (2005) reported the SGR of seabass was 0.98-1.19 %/day which was almost similar to the values in the present study. Biswas *et al*. (2010) reported the SGR of seabass was 4.93-5.07 %/day when conducted a research on optimization of feeding frequency of Asian seabass fry reared in net cages under brackish water environment which were invariably higher than the values in the present study. The FCR ranged 2.68-2.98 of the present study which are invariably lower than the FCR range 33.97-66.55 of seabass reported by Daet (2019) who conducted research on seabass in hapa-in-pond with different stocking density. Biswas *et al*. (2010) reported the FCR of seabass raged 2.23-3.04 when conducted a research on optimization of feeding frequency of Asian seabass fry reared in net cages under brackish water environment which are almost similar to the values in the present study.

The gross and net production ranged 1139.02-1460.78 kg/ha and 946.96-1255.55 kg/ha respectively among the different treatments of the present study. Monwar *et al*. (2013) recorded 899.26-1168.50 kg/ha production after 3 months culture period which are higher than the production of present study. Singh *et al*. (2012) stated that the stocking density of 1500 individual/ha was found to be superior as it gave the highest yield of seabass (541.50 kg/ha/yr)

as compared with 1000–2000 individual/ha stocking density, which could yield only 80.44 kg/ha/yr and 350.01 kg/ha/yr, respectively. The forage fish (Tilapia) should be in the ratio of 1:6 of seabass to tilapia for higher growth of seabass. The present investigation showed that specific growth rate (SGR) (%/day), gross and net yield (kg/ha) were obtained significantly higher by culturing Asian seabass at stocking density (25 individual/decimal) both in brackish and freshwater earthen ponds. Besides, the SGR, gross and net yield of seabass were higher in treatments conducted in brackish water than those of freshwater. To meet the increasing demand of animal protein as well as to raise the economic progress of fish farmers, the policy makers should take necessary steps to extend the culture techniques of seabass with artificial feed for the increase of fish production especially in the south-west coastal areas of Bangladesh.

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