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Spotted snakehead, *Channa punctata* (Bloch, 1793), stocking density effects on water quality, live food, and growth performances in Indian major carp polyculture system

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Abstract
Experiment conducted to examine the effect of stock densities of *Channa punctata* on water quality parameters, live food production, and growth performance in Indian major carps polyculture system at 12 earthen ponds (area: 200 m^2, 1.5 m depth) for 120 days. Stocking densities of *C. punctata*, 0 (T_1), 2000 (T_2), 3000 (T_3), and 4000 (T_4) individual ha^{-1} and rohu (*Labeo rohita*), silver carp (*Hypophthalmichthys molitrix*), catla (*Catla catla*), and mrigal (*Cirrhinus cirrhosis*) at the constant rate of 3000, 2500, 2000 and 2000 individual ha^{-1}, respectively with three replications. The mean initial weight of spotted snakehead and carps fingerlings were 1.50±0.07 g and 7.86±0.03 g, respectively; fingerlings fed commercial pellets twice daily. Water quality parameters were significantly different in all treatments. Zooplankton and benthos decreased with increasing stocking density of fish. The mean specific growth rate of carps was not significantly different with and without *C. punctata*. Food conversion ratio was low in T_1, while fingerlings survivability range was 67% to 89% in all treatments. The gross production of *C. punctata* in T_3 was the highest (143.53 kg ha^{-1}) followed by T_4 (123.26 kg ha^{-1}), and T_2 (97.66 kg ha^{-1}). The combined total production of spotted snakehead with carps was the highest in T_2 (1836.95 kg ha^{-1}) compared with T_3 (1760.53 kg ha^{-1}), T_1 (1806.18 kg ha^{-1}), and T_4 (1621.46 kg ha^{-1}). Overall, maximum yield of T_2 reflected proper feed utilization and appropriate species combinations.

Keywords: *Channa punctata*, carnivorous, fish culture, small indigenous fish species, bottom feeder

1. Introduction

One of the valuable small indigenous fish species (SIS) is the spotted snakehead *Channa punctata*, a maximum length about 25 cm, [1] distributed throughout the South-East Asian countries including Bangladesh and commonly found in waterbodies like ponds, rivers, canals, streams, and flood plains [2]. So far, these waterbodies are the main sources of *C. punctata* production which are decreasing day by day as a consequence of destruction of feeding, breeding, and nursery ground [3]. On the other hand, Indian major carps for instance rohu (*Labeo rohita*), catla (*Catla catla*), mrigal (*Cirrhinus cirrhosis*) and exotic carp silver carp (*Hypophthalmichthys molitrix*) are dominating as aquaculture species in polyculture system in Bangladesh, while less attention is paid toward many SIS including this spotted snakehead. In the carp polyculture, bottom feeder *C. punctata* are discarded from waterbodies as trash fish giving the blame of carnivorous and cannibalistic characteristics despite its popularity [4]. The key properties for its popularity are special taste, market price, nutritive value and low intramuscular bones along with medicinal uses in the form of special diet serve during convalescence, reduction of post-surgery pain, and rapid wound healing etc [5]. Moreover, the most important characteristics for adaptation hostile aquatic environment are its hardiness and air breathing which is suggesting the potentialities as a candidate for polyculture.

Polyculture, either native-exotic carps combination or carps with SIS, is a commonly culture method in Bangladesh. It provides significant contribution in total fish production of the country with the aim utilizing all-natural food present in the pond [6]. In this system, appropriate species composition and stocking density are the factors that determine production output to a great extent. Silver carp feeds on microscopic phytoplankton which prevents sudden algal blooms and increases food resources for benthophagous fish through its faecal pellet [7]. Catla is a surface feeder mainly feed on zooplankton [8], and it shares the upper feeding niche of the pond with the silver carp, while rohu and mrigal prefer plant matter including decaying plant materials [9]. Rohu is known as a mid-surface or water column feeder and mrigal is a bottom feeder. So, each species assumed to consume feed in its own niche and does not compete with others in polyculture system [10].

In any culture system, fish growth, appetite, metabolism, immunity, survivability, water quality, and yield etc. are directly or indirectly associated with stocking density [11, 12]. However, higher stocking densities can lead potential source of stress, but it could facilitate more profit by increasing biomass as well. Researchers are still searching appropriate stocking density for any fish species. Several researchers worked on the species combination of Indian major carps with SIS. So far, polyculture of spotted snakehead with major carps in the captive condition has not been reported. Before introducing of *C. punctata* into the polyculture of Indian major carps, the relationship between stocking density and yield is an important criterion that need to be established first. Therefore, the aim of this study was to examine the effect of stock densities of *C. punctata* on water quality parameters, live food production, and growth performance in Indian major carps polyculture system.

2. Materials and methods

2.1 Pond Preparation

The experiment was carried out at twelve earthen ponds (area: 200 m^2 with an average depth of 1.5 m) in Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. Experimental ponds are prepared following: dewatering, removing aquatic flora and fauna. All ponds exposed to sunlight with necessary renovation work. Ponds were applied with lime ($CaCO_3$) and fertilized with semi decomposed cow dung according to Rahman *et al.* [10].

2.2 Stocking fingerlings

Fingerlings of each species were collected from local suppliers and cultured for a period of 120 days. Spotted snakeheads were stocked in four treatments: T_1 (control), T_2, T_3, and T_4 with 0, 2000, 3000, and 4000 individual ha^{-1}, respectively. Rohu (*L. rohita*), catla (*C. catla*), silver carp (*H. molitrix*), and mrigal (*C. cirrhosis*) were stocked in each treatment at the rate of 3000, 2000, 2500, and 2000 individual ha^{-1}, respectively. There are three replications for each treatment. During stocking, mean body weight of spotted snakehead (1.50 ±0.07 g), rohu (8.00 ±0.28g), mrigal (8.98 ±0.25g), silver carp (7.15 ±0.12g), and catla (7.35 ±0.10 g) were calculated. Average stocking weight of carps fingerlings were 7.86±0.03 g in all treatments.

2.3 Culture management

Fingerlings were fed with commercial pellet (Naurish Feed. Ltd, Bangladesh: crude protein 35%, moisture 12%, lipid 3%, fiber 10% and ash 14%) at the rate of 5% of their body weight for the starting month, and declined gradually to 3% body weight at the end of the culture period in twice a day (at 09.00 and 16.00). Weighing 10% of total fish stock were measured to adjust the feeding rate in every month. Ponds were fertilized regularly in seven days intervals with cow-dung at 125 kg ha^{-1}, urea and triple super phosphate each at a rate of 6.25 kg ha^{-1} to maintain steady natural food abundance especially plankton and benthos.

2.4 Water quality parameters, plankton, and benthos enumeration

Water temperature, dissolved oxygen (Dissolved Oxygen Meter, YSI Model 58, Yellow Springs, OH, USA), transparency (Secchi disc), and pH (EC 10 portable pH meter, HACH, Loveland, Co, USA) of all ponds were measured on weekly basis. Nitrogen compounds (NH_3-N, NO_2-N, and NO_3-N) and phosphate phosphorus (PO_4-P) were measured bi-weekly by using a HACH kit (DR 5000, HACH). Water samples in plastic bottles (volume of 200 ml) were collected between 09:00 to 10:00 am in each sampling day. For plankton analysis, 10 L of pond water was collected monthly at five randomly selected locations and depths of each pond and filtrated through a 25 µm meshed plankton net. Each filtered was then transferred to a measuring cylinder and made up to a standard volume of 50 mL with distilled water and formalin to obtain a 10% buffered formalin solution and preserved in a sealed plastic bottle until examination. Plankton numbers were counted using a Sedgwick-Rafter counting cell (S-R cell) under a binocular microscope (Olympus, M-4000D, Tokyo, Japan) following Stirling [13]. Identification of plankton to the genus level was carried out using the keys from Bellinger [14]. Benthos from each pond bottom were collected three randomly selected places using a 225 cm^2 Ekman dredge in every month. Mud samples were washed through a sieve (250 µm mesh size) and macro-benthic organisms were separated and preserved in 10% buffered formalin. The microbenthic animals were then identified and counted following the standard method [13].

2.5 Final harvest

All ponds were harvested separately by repeated netting and complete dewatering on day 120. All fish harvested from each pond were counted, measured and weighed individually. Net production was determined by deducting the stocked biomass from the gross production (harvested biomass). Specific growth rate (SGR), feed conversion ratio (FCR), and survival rate of fish was calculated as follows:

$$SGR=\frac{\ln(\text{Final weight})-\ln(\text{Initial weight})}{\text{culture period (days)}}\times 100$$

Feed conversion ratio (FCR) was estimated based on harvested sample biomass as follows:

$$FCR=\frac{\text{Weight of feed used}}{\text{Weight of fish harvested}}\times 100$$

$$\text{Survival rate}=\frac{\text{Total no. of fish harvested}}{\text{Total no.of fish stocked}}\times 100$$

2.6 Statistical analysis

The results were presented as mean ± standard deviation. The data were compared among treatments by One-way analysis of variance (ANOVA) using statistical package for social science (SPSS version11.5) software. Water quality parameters, plankton, and benthos data were compared in a repeated measure analysis; however one-way ANOVA was also performed. When the mean difference was significant ($P< 0.05$), Tukey's test was used to compare the mean among all the treatments.

3. Results

3.1 Water quality parameters

The physico-chemical parameters of water sample in 12 earthen ponds were significantly varied in all treatments except for temperature, pH, and ammonia nitrogen (Table 1). The temperature remained almost at around 30 ^{0}C in all treatments.

Mean transparency differed significantly among the treatments, the highest in the T_1 (32.89 cm). Dissolved oxygen was higher in T_1 and T_2, followed by T_3 and T_4. The water nutrients (nitrite, nitrate, and phosphate) were varied significantly (P< 0.05) among the treatments.

Table 1: Mean (±SD) values of water quality parameters recorded from different treatments.

Water parameter	Treatment			
	T_1	T_2	T_3	T_4
Temperature (0C)	30.31±1.47a	30.34±1.52a	30.43±1.46a	30.39±1.50a
Transparency (cm)	32.89±0.78a	32.15±0.66a	29.73±0.51ab	27.06±1.07b
Dissolved Oxygen (mg L-1)	8.12±1.79a	8.07±1.65a	6.20±1.32ab	5.66±0.75b
pH	7.6±0.12a	7.8±0.49a	8.1±0.21a	8.2±0.27a
Ammonia-N (mg L^{-1})	0.41±0.06a	0.43±0.05a	0.42±0.07a	0.36±0.06a
Nitrite-N (mg L^{-1})	0.020±0.007b	0.028±0.011ab	0.026±0.011ab	0.085±0.081a
Nitrate-N (mg L^{-1})	0.15±0.04b	0.23±0.06ab	0.22±0.09ab	0.27±0.09a
Phosphate-P(mg L^{-1})	0.78±0.32ab	1.09±0.37a	0.45±0.17b	0.53±0.20b

Means with different superscript letters in the same row are significantly different (P<0.05) among treatments. T_1 = control, spotted snakehead 0 individual ha^{-1}, T_2 = treatments spotted snakehead 2000 individual ha^{-1}, T_3 = spotted snakehead 3000 individual ha^{-1}, and T_4 = spotted snakehead 4000 individual ha^{-1}.

3.2 Life food biomass (Plankton and benthos)

The overall mean of plankton (phytoplankton and zooplankton) counts in T_1, T_2, T_3, and T_4 were 64521, 65593, 65941, and 60852 individuals L^{-1}, respectively (Table 2). Phytoplankton represented about 83-87% of the total plankton communities. Four groups of phytoplankton, and two groups of zooplankton were identified. Chlorophyceae (14 genera) was the dominant group followed by Bacillariophyceae (8 genera), Cyanophyceae (7 genera), and Euglenophyceae (2 genera) in the experimental ponds. There was no significant (P< 0.05) difference in total numbers of phytoplankton among all treatments (Figure 1).

Zooplankton communities were consisted of Rotifera (6 genera) and Crustacea (5 genera). Mean abundance of plankton as a whole did not vary significantly in all treatments except T_4, but zooplankton differed significantly with a higher mean value in T_1 (10691±2.23) followed by T_2 (9062±1.42), T_3 (8314±1.84), and T_4 (6722±1.52) individuals L^{-1}, respectively. The abundance of zooplankton decreased with increasing the stocking density of spotted snakehead and time of culture period (Figure 2). The zooplanktons mean markedly varied (P< 0.05) in all the treatments.

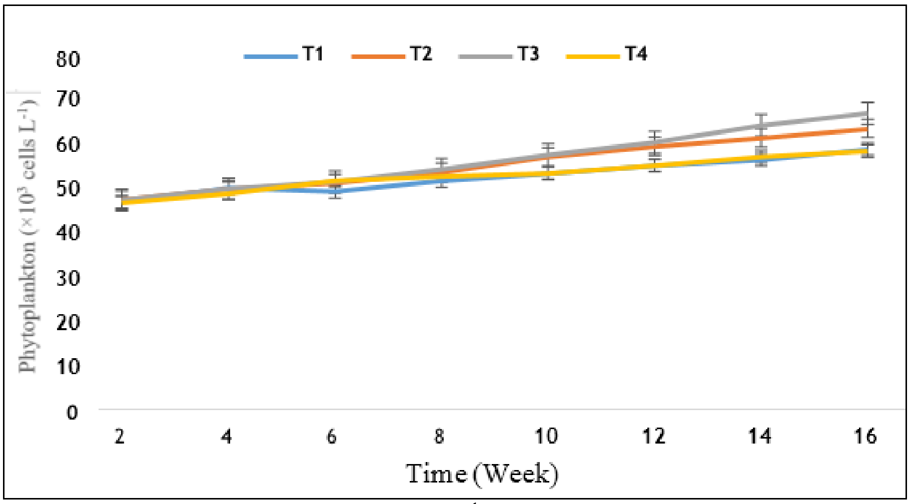
Table 2: Plankton abundance (×10^3 cells L-1) in all treatments (mean ±SD).

Parameter	Treatment			
	T1	T2	T3	T4
Phytoplankton (×10^3 ind. L-1)	53.83±4.04a	56.53±5.95a	57.65±7.09a	54.13±3.99a
Zooplankton (×10^3 ind. L-1)	10.69±2.23a	9.06±1.42a	8.31±1.84ab	6.72±1.52b
Total plankton	64.52±1.41a	65.59±2.0 a	65.96±0.21a	60.85±1.45b
% of phytoplanton	83	86	87	89
Benthos (ind. m-2)	41.81±4.35a	41.02±3.85a	34.69±6.03ab	32.25±7.04b

Means with different superscript letters in the same row are significantly different (P<0.05) among treatments. T_1 = control, spotted snakehead 0 individual ha^{-1}, T_2 = treatments spotted snakehead 2000 individual ha^{-1}, T_3 = spotted snakehead 3000 individual ha^{-1}, and T_4 = spotted snakehead 4000 individual ha^{-1}.

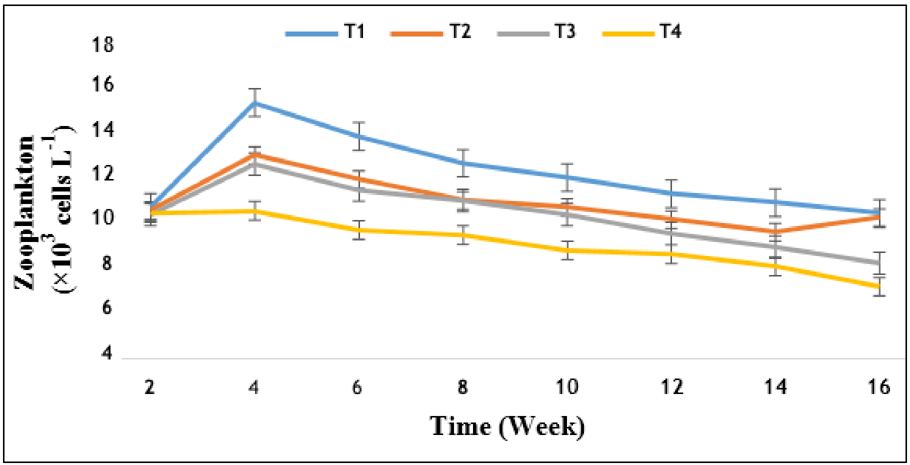
There were treatment effects on the mean abundance of macro-invertebrates in the pond bottom. The mean of macro-benthos density ranged from 22.2 to 44.57 individuals m^{-2} in different treatments (Figure. 3).

The dominant group was Chironomidae (55.12%) followed by Oligochaeta (20.5%), Mollusks (15.34%), and unidentified (9.1%). The highest benthos abundance was found in T_1 and T_2 compared with T_3 and T_4. While, there was no significant (P< 0.05) difference in T_1 and T_2 but varied significantly in other treatments. The benthos abundance reduced significantly in the treatment having spotted snakehead in higher densities because *C. punctata* is a bottom feeder.



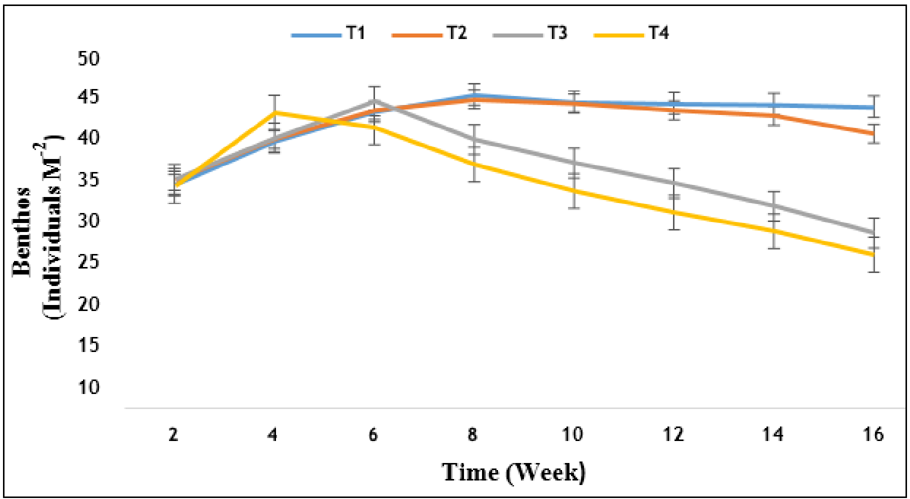
T_1 = control, spotted snakehead 0 individual ha^{-1}, T_2 = treatments spotted snakehead 2000 individual ha^{-1}, T_3 = spotted snakehead 3000 individual ha^{-1}, and T_4 = spotted snakehead 4000 individual ha^{-1}.

Fig 1: Biweekly abundance of phytoplankton among the treatments.



T_1 = control, spotted snakehead 0 individual ha^{-1}, T_2 = treatments spotted snakehead 2000 individual ha^{-1}, T_3 = spotted snakehead 3000 individual ha^{-1}, and T_4 = spotted snakehead 4000 individual ha^{-1}.

Fig 2: Biweekly abundance of zooplankton among the treatments.



T_1 = control, spotted snakehead 0 individual ha^{-1}, T_2 = treatments spotted snakehead 2000 individual ha^{-1}, T_3 = spotted snakehead 3000 individual ha^{-1}, and T_4 = spotted snakehead 4000 individual ha^{-1}.

Fig 3: Biweekly abundance of benthos among the treatments.

3.3 Growth Performance

The final individual weight, mean weight gain of harvested *C. punctata* was significantly higher at T_3, and T_2 than T_4 treatment, while T_2 and T_3 did not varied significantly, but those of carps showed high in T1 and T2 compared to other treatments (Table 3). The SGR of *C. punctata* was significantly lower in the T_4 (2.88%) than in the T_2 (3.01%) and T_3 (3.09%).

The survival of *C. punctata* was also higher in the T_2 (89%) than treatments T_3 (81%) and T_4 (67%). There was no

significant (P<0.05) differences of SGR between T_2 and T_3 treatments. Higher FCR observed in the T_4 where the density of fish species was the highest, while lower FCR was found in the T_1 where the density of fish species was the lowest.
The gross yield of *C. punctata* was 1.5 and 1.3 times higher in the treatments T_3, and T_4, respectively than T_2; that of carp was higher in the T_1 than other treatments. The combined gross production of all fish species was high significantly (P<0.01) in the T_2 and low in the T_4, while no significant (P<0.05) different between the T_2 (1836.95 kg ha^{-1}), and the T_1 (1806.18 kg ha^{-1}) treatment.

Table 3: Effects of spotted snakehead stocking density and carps on growth and yield parameters (mean ±SD) of spotted snakehead and carps

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Spotted snakehead				
Initial mean weight (g)		1.50±0.07a	1.50±0.17a	1.5±0.11a
Final mean weight (g)		54.65±0.64a	58.69±0.41a	46.00±0.28b
Mean weight gain (g)		53.18±0.68a	57.25±0.50a	44.54±0.22b
% Weight gain		3606.70±32.00a	3983.60±36.11a	3052.70±10.78b
Survival rate (%)		89.35±0.33a	81.52±0.37b	66.99±0.46c
Specific growth rate (% bw d^{-1})		3.01±0.03a	3.09±0.05a	2.88±0.02b
Gross Production (kg ha-1)		97.66±4c	143.53±10a	123.26±5b
Carps				
Initial mean weight (g)	7.86±0.03a	7.86±0.01a	7.86±0.04a	7.86±0.01a
Final mean weight (g)	207.90±0.49a	206.71±0.6a	200.06±1.80b	194.65±1.41c
Mean weight gain (g)	200.05±0.48a	198.86±0.6a	192.18±1.82b	186.79±1.42c
% Weight gain	2548.40±1.53a	2531.60±11.10a	2440.40±29.74b	2374.90±20.21c
Survival rate (%)	91.45±0.64 a	88.57±0.58 b	85.08±0.64c	81.02±0.80 d
Specific growth rate (% bw d^{-1})	2.74±0.07 a	2.71±0.01a	2.70±0.02a	2.67±0.01a
Gross Production (kg ha-1)	1806.18±25a	1739.29±51a	1617.0± 69b	1498.2±76 c
Combined yield				
Food conversion ratio	1.36±0.12^a	1.41±0.21^a	1.52± 0.08^b	1.58±0.18^b
Gross Production (kg ha-1)	1806.18±0.25a	1836.95±0.51a	1760.53±0.69b	1621.46±0.76c

Means with different superscript letters in the same row are significantly different (P<0.05) among treatments. T_1 = control, spotted snakehead 0 individual ha^{-1}, T_2 = treatments spotted snakehead 2000 individual ha^{-1}, T_3 = spotted snakehead 3000 individual ha^{-1}, and T_4 = spotted snakehead 4000 individual ha^{-1}.

4. Discussion

4.1 Water quality parameters

Most water quality parameters were found acceptable for aquaculture. The water temperature (around 30°C) in the experimental ponds were within the acceptable for the rearing of fish fry and fingerlings as described by Rahman *et al.* [15]. Water transparency was observed a range from 27 to 33 cm in the present study; the acceptable range of transparency is 15 to 40 cm [16]. The lowest transparency was recorded in the T_4, possibly due to available phytoplankton and other soil particles. The similar results reported at earthen ponds in Bangladesh by Kohinoor *et al.* [17]. The experimental pond pH values were found a favorable for the grow-out phase of fish in pond culture [18]. Because nutrient availability and the primary production found optimum level at the neutral to alkaline range of pH, fish growth is expected to be better at this pH. Although the dissolved oxygen was reduced with increasing the stocking density of fish, the level of oxygen was acceptable in all the treatments. Similar trends of dissolved oxygen contents in various carp and barb nursery ponds were also reported by Rahman and Rahman [19] and Rahman *et al.* [15]. Nitrogenous compounds (ammonia, nitrite and nitrate) and phosphate were within the suitable limit throughout the study [18] for tropical aquaculture.
The abundance of total plankton was not different significantly in all treatments except T_4. Generally, a higher plankton number indicates higher productivity of the pond. Among the plankton communities, phytoplankton abundance was higher than zooplankton which might be due to heavy manuring with organic fertilizer (cattle dung), excess uneaten feeds [20] and a higher rate of supplementary feeding [21]. Phytoplankton counts increased after the second month and thereafter, showed almost stable trends up to the end of the study in all the treatments. This supports the fact that plankton-feeding fish, silver carp, was able to control phytoplankton in carp polyculture ponds. The grazing activities of silver carp might also stimulate phytoplankton production [22]. Similar results were reported in various carp and barb ponds too by Rahman and Rahman [19] and Rahman *et al.* [15]. The declining trend in the count of zooplankton in the experiment might indicated increasing consumption and utilization by *C. punctata* which mainly feed on zooplankton [8]. Plankton diversity was similar to the observations of Rahman *et al.* [23], who conducted studies in the earthen ponds of Bangladesh.

4.2 Production performance of *C. punctata* with major carps

Growth in terms of final weight and weight gain of major carp species were significantly higher ($P < 0.05$) in T_1 and T_2 than in T_3 and T_4, while *C. punctata* growth was higher ($P < 0.05$) in T_3 and T_2 than the values obtained in T_4. So, growth trends of carps were probably not affected when cultured with *C. punctata* in T_2 but slightly reduced in T_3 and T_4 suggesting proper feed utilization and low competition for space in the niche of *C. Punctata* [15]. The mean SGR values of carps in different treatments were not significantly different with and without *C. punctata*. Similar results were also obtained for carp-SIS culture by Wahab *et al.* [24], and Rahman *et al.* [15] whereby polyculture of different fishes was accomplished in ponds through feeding and fertilization. In the current experiment, significant differences ($P < 0.05$) among treatments were observed in the survival rates of major carps

(81-91%) and *C. punctata* (67-89%). However, the overall higher survival of all species indicated that *C. punctata* did not compete with other carps for food and habitat. A similar survivability rate was found in the mixed culture of Indian major carps with SIS [25], and in polyculture of mahseer *Tor putitora* with native major carps. The gross production of combined fish species was the highest in the T_2 and the lowest in the T_4, while T_1 and T_2 productivities were not significantly different. It might be due to the appropriate species ratios and combinations. So, this study combined culture of carps with *C. punctata* properly utilized supplementary and natural food especially phytoplankton and zooplankton. Similar trends of productions were also obtained by Rahman *et al.* [26] in which carp polyculture with prawn in earthen ponds. The highest production of combined all species was found in the T_2 showing no harmful effect of *C. punctata* on the growth performance, and productivity of major carps in the earthen ponds.

5. Conclusion

In the present study, *C. punctata* was experimentally introduced in major carps polyculture system to observe SIS effect on the production of carps. From the results, it might be concluded that the polyculture of *C. punctata* with major carps increased the total biomass production of all species due to presence of suitable water quality parameters, live food, proper species combination for food and space. Also, no negative effect of *C. punctata* was observed on carps production in respect of overall growth and production. Therefore, *C. punctata* should be considered as a suitable species for aquaculture in combination with carps specially in the earthen ponds polyculture system. This study represents the first successful attempt to culture the endangered *C. punctata* with major carps in captive condition. The findings of the present study would have greater implications for the development of aquaculture, management and conservation of this SIS of Bangladesh as well.

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