TOPIC AREA

MITIGATING NEGATIVE ENVIRONMENTAL IMPACTS

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ADVANCING SEMI-INTENSIVE POLYCULTURE OF INDIGENOUS AIR-BREATHING FISHES, KOI AND SHING, WITH MAJOR INDIAN CARPS FOR ENHANCING INCOMES AND DIETARY NUTRITION WHILE REDUCING ENVIRONMENTAL IMPACTS

Mitigating Negative Environmental Impacts/Experiment/16MNE01NC

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ABSTRACT

The use of Koi or climbing perch (*Anabas testudineus)* in aquaculture has grown substantially over the past decade in Bangladesh because it has a high market value and is rich in nutrients. Further, being an air-breathing fish, Koi have a strong capacity to tolerate poor oxygen environments. Koi production is currently limited to monoculture systems with intensive use of commercial-grade feeds. Feed constitutes almost 80% of the total costs for producing Koi and thus methods to reduce feed inputs can provide significant economic benefits, particularly if fish are co-cultured with carps that rely primarily on natural pond productivity rather than direct consumption of formulated feeds. Thus, the aim of our first study was to investigate growth and production of Koi when used in polyculture with major Indian carp species, Rui (*Labeo rohita*) and *Catla* (*Catla catla*) relative to that observed with Koi monoculture. We also examined the effects of combining reduced feed ration and pond fertilization on Koi-carp polyculture. The experiment consisted of four treatments, with three replicates each (12 ponds; 100 m^2 area, 1.5 m depth). T1 consisted of a Koi monoculture (5/m^2) with full daily feeding while the other three treatments consisted of a Koi-carp polyculture (Catla, 0.2/m^2; Rui, 0.8/m^2; Koi, 5/m^2) with full daily feeding (T2), 75% daily feeding (T3), or 50% daily feeding (T4). Additionally, the ponds for T3 and T4 were fertilized weekly with urea and triple super phosphate (28 kg/ha N, 5.6 kg P/ha) to boost pond productivity. Koi were fed a full daily ration of commercial feed (CP feed) according to current practice (20% down to 5% body weight/day) or a fraction of this based on treatment groups. Growth and production of Koi was greater in polyculture with carps (T2-4) than in monoculture and production increased with decreasing feed ration. Thus, the highest production of Koi was in T4 (3484 kg/ha) and the lowest production was in T1 (1963 kg/ha). No significant differences were observed in growth or production of Rohu, although both were slightly higher in T4, while Catla production was significantly lower in T3 (393 kg/ha) relative to T2 (716 kg/ha) and T4 (634 kg/ha) likely due to the lower specific growth rates and survival rates for T3. Total production, net return, and benefit cost ratio were all greatest in T4, which employed a 50% reduction in feed, and then decreased with increasing feed ration. Overall, combining the culture of Koi with carps is more economical and can increase production and earnings for fish farmers in Bangladesh. Additionally, reducing the amount of feed applied to the ponds by 50% mitigates production costs and enhances feed conversion which can further increase food availability and incomes for rural farming households.

The stinging catfish or shing (*Heteropneuestes fossilis*) is another high value, micronutrient dense, air-breathing fish that has a strong capacity to tolerate poor oxygen environments and thus culture of Shing has also been increasing in Bangladesh. In a second study, we investigated the effects of combining Shing at different stocking densities into Koi-carp polyculture. This study consisted of four treatments with three replications. T1 consisted of a Koi-carp polyculture without Shing (Catla, $0.2/m^2$; Rohu, $0.8/m^2$; Koi, $5/m^2$), while T2-4 contained Shing stocked at the densities of $1.0/m^2$, $2.0/m^2$, and $3.0/m^2$, respectively. A reduced 50% feed ration (10% down to 1.5% body weight/day) was applied to the ponds based on the biomass of both Koi and Shing which was adjusted every two weeks from fortnightly sampling of fish weights and all ponds were fertilized weekly. Production and survival of Koi, Rohu, and Catla was higher in ponds containing lower stocking densities of Shing (T2 and T3). Gross production and returns were greatest in T4 because of the increased abundance and higher market value of Shing. However, due to the higher cost of feed and lower survival rates for all species in T4, the greatest net profit and benefit cost ratio was observed in T3 indicating that incorporating Shing into Koi-carp polyculture at a stocking density of $2.0/m^2$ would be the most beneficial for increasing food production and incomes for rural farmers in Bangladesh.

INTRODUCTION

Air-breathing fishes provide a significant advantage for pond culture, as they tend to be resilient to harsh conditions, particularly during periods of low-oxygen, which can occur with high temperatures, drought, or poor water quality. Indigenous air-breathing fishes, such as Shing catfish (stinging catfish, *Heteropneustes fossilis*) and Koi (climbing perch, *Anabas testudineus*) are commonly found in open waters, paddy fields, and swamps of Bangladesh. Because of accessory respiratory organs they can even survive for a few hours out of the water. These fishes have been successfully cultivated in Bangladesh in recent years and command a high market value (DOF, 2012; Kohinoor et al., 2011), 3-7 times that of other commonly cultured finfishes (striped catfish or *Pangasius* and tilapia). Both are currently in great demand by consumers for their taste and nutritional value (Hasan et al., 2007, Vadra, 2012; Vadra and Sultana, 2012). Shing catfish is particularly high in both iron (226 mg 100 g^{-1}) and calcium relative to other freshwater fishes and has been recommended in the diets of the sick and convalescent (Saha and Guha, 1939; Singh and Goswami,1989). Culture of these indigenous species with high mineral content is an important step for increasing the yield and diversity of aquaculture products for consumption in Bangladesh and in reducing some types of dietary malnutrition, such as iron-deficient anemia (Dey et al., 2008; Micronutrient Initiative/UNICEF, 2004).

Production of Shing and Koi is currently limited to monoculture systems with high stocking densities and intensive use of commercial-grade feeds (30-35% crude protein). As feed can comprise a majority of total production costs (> 70%), there is limited participation by small homesteads utilizing the current practices for these fish and thus creating a significant impediment to further expansion of this industry. Further, the use of high-levels of feed inputs has led to a persistent deterioration of pond water quality (eutrophication; cf. Chakraborty and Mirza, 2008; Chakraborty and Nur, 2012) and periodic mass mortalities and disease outbreaks. As most ponds are located near homesteads and villages, poor water quality and foul odors related to greater nutrient-loading impacts both local health and socio-economic tensions within the community (personal communication, Nural Amin, local farmer in Tarakanda, Mymensigh, July, 2012). Through field visits to Mymensingh, this research team observed firsthand that most air-breathing fish farms are often overfed, thus some of the problems associated with farming of air-breathing fishes can be alleviated through better management and implementation of semi-intensive culture practices. These problems may also be mitigated through polyculture, where excess nutrients and algae can be utilized by other species, for instance carps that feed primarily on plankton.

To this end, in Phase I of our project we evaluated whether carps could be incorporated into pond culture of Shing catfish. We found that addition of indigenous Indian carps (Rohu and *Catla*) enhances total fish yields and nutrient utilization of feed inputs over that seen with Shing monoculture alone. Shing growth was little impacted by culture with carps. Moreover, we found that reducing ration levels by as much as 50% from those currently used by the farming community (*e.g.* 20-5% body weight/day) provides additional return on investment of almost 100% in Shing-carp polyculture. We also demonstrated that Koi could be successfully cultured with either *Catla* alone or with *Catla* and Rohu under the reduced feeding ration established for Shing. However, our studies did not compare Koi-carp polyculture with Koi monoculture or whether the 50% reduction in feed inputs utilized produces similar growth and fish yields as could be seen with feeding at a higher rate. Therefore, we first assessed whether mixed trophic polyculture of Koi and carps is a better technology than Koi monoculture and whether feed reductions can produce equivalent or better production yields and can improve nutrient utilization and water quality over current feeding practices.

Recent studies using high stocking densities (25-37 fish/m^2) and prohibitively high feed inputs (100% down to 5% body weight/day) suggests that both Shing and Koi can be cultured together (Chakraborty and Nur, 2012). We propose to extend the new semi-intensive Koi-carp technology developed here to evaluate whether Shing might provide additional increases in fish yields and returns on investment in Koi-carp polyculture. Indeed, farmers are now interested in understanding if culture of both air-breathing fishes with carps might provide economic advantages, particularly under a reduced feed ration. Here we assessed the addition of Shing stocked at different densities in Koi-carp growout. To our knowledge, the incorporation of Shing, Koi, and carps in polyculture has yet to be evaluated and this could represent an additional technology for enhancing efficiency of food production in ponds, yield of nutritious fish, and farmer incomes.

OBJECTIVES

1. Compare combined polyculture of Koi with two major carps (Rohu and *Catla*) versus Koi monoculture under semi-intensive pond culture conditions.
2. Assess the effect of reduced feed ration in polyculture of carps and Koi. This study will identify a feed-reduction ration needed for equivalent or better production yields through increased nutrient utilization efficiency and impacts on the environmental water quality.
3. Assess economic and environmental benefits of combining Shing with Koi-carp pond polyculture.
4. Evaluate overall performance and economic returns of the improved management strategies.

MATERIALS AND METHODS

Location

These studies was performed onsite at the Fisheries Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Water quality analysis was performed at the Water Quality and Pond Dynamics Laboratory (BAU).

Pond Preparation

Prior to initiating each study, the ponds at the BAU Fisheries Field Laboratory (100 m^2, 1.5 m depth) were dried, re-excavated, and limed (25 g $CaCO_3$/m^2). Ponds were then fertilized with 28 kg N/ha as urea and 5.6 kg P/ha as triple super phosphate (TSP) prior to being stocked with the appropriate species.

Study 1- Assess reduced ration levels for combined polyculture of two major carps (Rohu and Catla) with Koi

This study evaluated the effects of 75% and 50% daily rations on growth, production yield, and economic returns for semi-intensive Koi-carp polyculture. Four different treatments were evaluated as outlined in Table 1 and each treatment was replicated in 3 separate ponds. The initial stocking weights were 0.69 ± 0.08 g, 21.76 ± 7.76 g, and 32.27 g for Koi, Rohu, and *Catla*, respectively. During the experimental period, ponds belonging to T3 and T4 were fertilized weekly at a rate of 28 kg N/ha (urea) and 5.6 kg P/ha (TSP) while T1 and T2 did not receive any fertilizer treatments. Ponds in T1 and T2 were given full daily rations of a floating commercial feed (30% crude protein) at the rates currently employed by farmers (20% bw/day, 0-30 days; 15% bw/day, 31-60 days; 10% bw/day, 61-90 days; 5% bw/day, > 90 days) while T3 ponds received 75% daily rations and T4 received 50% daily rations. The percentage of feed applied to each pond was based on the biomass of Koi alone. Water quality parameters (temperature, transparency, etc.) were measured fortnightly while plankton and benthos samples were collected fortnightly and monthly, respectively. All ponds were sub-sampled every 15 days for growth measurements and upon study completion or after 126 days the specific growth rate (SGR) for each species was calculated. Total feed conversion ratios (FCR) and cost-benefit analyses were also calculated at the end of the study.

Table 1. Experimental design for Study 1.

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Rohu (*L. rohita*)	0	80 (0.8/m^2)	80 (0.8/m^2)	80 (0.8/m^2)
Catla (*C. catla*)	0	20 (0.2/m^2)	20 (0.2/m^2)	20 (0.2/m^2)
Koi (*A. testudineus*)	500 (5.0/m^2)	500 (5.0/m^2)	500 (5.0/m^2)	500 (5.0/m^2)
Fertilization (/ha)	0	0	28 kg N, 5.6 kg P	28 kg N, 5.6 kg P
Daily Feeding	100% Ration	100% Ration	75% Ration	50% Ration
Replicates (*n*)	3	3	3	3

Study 2- Effect of combining Shing at different stocking densities in Koi-carp polyculture

This study assessed whether Shing catfish could be incorporated into Koi-carp polyculture to provide an additional crop of high nutritional and economic value for farmers. Three different stocking densities of Shing were evaluated (T2- 1.0/m^2, T3- 2.0/m^2, T4- 3.0/m^2) and compared to a control Koi-carp culture with no Shing (T1, Table 2). Koi, Rohu, and *Catla* were stocked at the same densities from Study 1. During the experimental period, ponds were fertilized at a rate of 28 kg N/ha/week (urea) and 5.6 kg P/ha/week (TSP) and each pond received a 50% daily feed ration (10% based on the biomass of Koi and Shing in each treatment. Water quality parameters (dissolved oxygen, pH temperature, transparency, ammonia, nitrates, nitrites, phosphates, alkalinity, and chlorophyll-a) were measured fortnightly. All ponds were sub-sampled every 15 days for the collection of growth data. Upon conclusion on the study at 140 days, SGR, FCR, and production yields were calculated and a cost-benefit analysis performed.

Table 2. Experimental design for Study 2.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Rohu (*L. rohita*)	80 (0.8/m^2)	80 (0.8/m^2)	80 (0.8/m^2)	80 (0.8/m^2)
Catla (*C. catla*)	20 (0.2/m^2)	20 (0.2/m^2)	20 (0.2/m^2)	20 (0.2/m^2)
Koi (*A. testudineus*)	500 (5.0/m^2)	500 (5.0/m^2)	500 (5.0/m^2)	500 (5.0/m^2)
Shing (*H. fossilis*)	0	100 (1.0/m^2)	200 (2.0/m^2)	300 (3.0/m^2)
Fertilization (/ha)	28 kg N, 5.6 kg P	28 kg N, 5.6 kg P	28 kg N, 5.6 kg P	28 kg N, 5.6 kg P
Daily Feeding	50% Ration	50% Ration	50% Ration	50% Ration
Replicates (*n*)	3	3	3	3

Equations
SGR (% bw d^{-1}) = [{Ln (final weight) – Ln (initial weight)} ÷ Culture period in days] × 100
Survival (%) = (Number of fish harvested ÷ Number of fish stocked) ×100
Gross production = Number of fish harvested × Final weight of fish
FCR = Amount of feed applied to pond ÷ Total fish weight gain

Statistical Analysis
All growth and economic parameters were analysed with a one-way ANOVA and Tukey's HSD post-hoc test (JMP 13).

RESULTS AND DISCUSSION

Study 1- Assess reduced ration levels for combined polyculture of two major carps (Rohu and Catla) with Koi

This experiment consisted of four treatments that aimed to determine whether reduced feeding rations can be successfully applied to Koi-carp polyculture. All water quality parameters (measured fortnightly) were deemed suitable for fish culture throughout the study period and did not differ significantly between treatments (Table 3). We identified 28 genera of phytoplankton and 12 of zooplankton over the course of the study period in all ponds, however no differences in total plankton abundance were observed (Table 4). In addition to plankton, we measured the abundance of benthic organisms in each treatment as these can also serve as a food source for carps. Species were grouped into four classifications (oligochaeta, chironomidae, Mollusca, or unidentified) and total abundance was also calculated. Although not statistically significant, the Koi monoculture (T1) had a greater overall abundance of benthic organisms relative to the combined Koi-carp cultures, likely due to the consumption of these organisms by the two carp species. Further, the abundance had a tendency to decrease with decreasing feed ration suggesting that the fish may be increasing their consumption of natural food sources within the ponds to compensate for the reductions in commercial feed.

Although there were no significant differences in growth or production between treatments for Rohu, production was slightly higher in T4 which provided the 50% reduced feeding ration (Table 5). Catla production was significantly lower in T3 (393.0±33.8 kg/ha) relative to T2 (715.5±32.8 kg/ha) and T4 (634.1±26.4 kg/ha) which is likely due to both the lower specific growth rates and the lower survival rates in this group (Table 5). Koi growth and production was higher in polyculture (T2-4) than in the monoculture (T1, 1963.2±5.87 kg/ha) and tended to increase with decreasing food ration (Table 5). Thus, production was greatest in T4 (3484.0±37.8 kg/ha), followed by T3 (3311.0±215 kg/ha) and T2 (2311.6±38.4 kg/ha). T4 also exhibited the highest total production for all species and the best feed conversion ratio (Table 5), indicating that current guidelines overestimate the amount of feed required and a 50% reduction combined with pond fertilization to increase natural productivity could reduce the costs of fish culture. Indeed, T4 provided the greatest net return (540,772±28185 BDT/ha) and benefit cost ratio (2.18) relative to all other treatments (Table 6). The second highest return was in T3 (400,122±43361 BDT/ha), followed by T2 (266,644±12670 BDT/ha), and T1 (76,051±1056 BDT/ha). Our results indicate that the addition of Koi to carp polyculture enhances production of this species which is beneficial for the fish farmers as Koi fetch a higher market value than carps. Further, we have shown that reducing feed rations by 50% in Koi-carp polyculture enhances feed conversion ratios, production, and profits. Thus, adopting these practices including feeding at a rate range of 10%-2.5% body weight/day could increase fish production, food availability and incomes for rural fish farmers in Bangladesh.

Study 2. Effect of combining Shing at different stocking densities in Koi-carp polyculture
This study assessed the impact of adding Shing at different densities to Koi-Carp polyculture. Ponds were fertilized throughout the study to provide primary productivity for carps, while a 50% feed

ration was applied based on the biomass of Koi and Shing. All water quality parameters were deemed suitable for fish culture throughout the study period and differed only slightly between treatments (Table 7). Phytoplankton, zooplankton, and total plankton levels were all higher in T3, suggesting greater productivity within these ponds, although only the increase in zooplankton was significant (Table 8). Benthic organism abundance was also evaluated and determined to be highest overall in T1 (1562.69±8.08) and T2 (1369.00±10.5), followed by T3 (1198.35±51.9), and lowest in T4 (941.56±85.7) (Table 8). This could indicate that these organisms are being consumed by both the Shing and carps or that the presence of Shing within the ponds prevents these organisms from flourishing.

The highest gross production yield for Koi was observed in T2 (3634.17±9.22 kg/ha), followed closely by T3 (3594.27±9.33 kg/ha) and T4 (3547.92±11.72 kg/ha) with T1 being the lowest (3222.71±6.17 kg/ha). This is likely due to the higher survival rate in T2 as both T3 and T4 had higher specific growth rates (Table 9). For Rohu, the survival rate and gross production yield was greatest in T3 (93.5%, 1165.95±1.23 kg/ha) and lowest in T4 (82%, 1043.31±3.48 kg/ha). Similarly, Catla survival and production was greatest in T2 (92%, 452.79±0.42 kg/ha) and T3 (90%, 443.18±0.44 kg/ha) but lowest in T4 (79%, 389.47±0.32 kg/ha). Together, this suggests that the addition of Shing at low stocking densities is beneficial to the production of Koi and carps but higher densities of Shing are detrimental for these species. Gross production of Shing increased with increasing stocking density and was thus highest in T4 (Table 9), however the survival rates of Shing were 68%, 72%, and 67% in T2-4, respectively, again suggesting that lower stocking densities may be more beneficial overall. Further, the overall feed conversion ratio was significantly higher in T4 (1.86) relative to all other treatments (T1- 1.62, T2- 1.59, T3- 1.53) (Fig. 4).

Overall production was related to the stocking density of Shing within the ponds. Thus, the highest yield was observed in T4 (7130.56±14.43 kg/ha), followed by T3 (6766.99±9.17 kg/ha), T2 (5887.26±10.37 kg/ha), and T1 (4696.98±6.85 kg/ha) (Fig. 5). T4 also exhibited the highest gross return as Shing fetch a much higher market price (450 BDT) than the other three species (Koi- 180 BDT, Rohu- 170 BDT, Catla- 150 BDT) (Fig. 6). However, due to the increased cost of feed and the lower survival rates for all species at the highest stocking density of Shing, T3 produced the greatest net return at 676, 069±1946 BDT and highest Benefit Cost Ratio (BCR) of 1.72 (Table 10). T4 produced the second highest return and BCR (662,829±3505 BDT, 1.56), followed by T2 (402,250±1959 BDT, 1.49) and T1 (161,360±1224 BDT, 1.24). This result indicates that the addition of Shing to Koi-carp polyculture at moderate stocking densities is beneficial and could increase incomes and food production for rural fish farmers in Bangladesh.

CONCLUSION

The results of this investigation indicate that Koi and Shing are ideal candidates for polyculture with carps. The production of Koi is significantly higher in polyculture with Rohu and Catla than in monoculture, and the addition of moderate stocking densities of Shing (2.0/m^2) further enhanced growth and production of Koi as well as both carp species. Our data also shows that a 50% reduction in commercial feed application (feeding at 10-2.5% bw/day versus 20-5% bw/day) using the stocking densities employed in this study combined with weekly pond fertilization to support natural productivity can lower production costs without any negative impacts on fish growth or survival. Nutrient overloading often leads to poor water quality and disease outbreaks and thus reducing the amount of feed applied to the ponds could have positive environmental and socio-economic impacts. Although Koi and Shing fetch higher market value and have a higher nutrient content than carps, they are currently limited to monoculture and have high production costs from the use of commercial feeds which has prevented smaller farming households from culturing these species. Thus, adding Koi and Shing to existing carp culture systems and reducing feed application could allow an overall increase in production of both species in Bangladesh as well as enhance earnings and food availability for small-scale rural fish farmers.

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TABKES AND FIGURES

Table 3. Average water quality parameters from Study 1. Values are mean ± SEM. No significant differences were observed between treatments. T1, Koi monoculture; T2-4, Koi-Rohu-Catla polyculture.

	T1 (100% feed)	*T2 (100% feed)*	*T3 (75% feed)*	*T4 (50% feed)*
Temperature (°C)	28.96±0.23	28.79±0.35	29.71±0.28	30.17±0.89
Transparency (cm)	36.58±2.53	32.75±3.05	36.92±3.68	31.25±2.97
Alkalinity (mg l^{-1})	85.50±7.05	79.33±6.24	78.67±6.22	84.75±6.75
pH	7.35±0.26	7.20±0.27	7.38±0.27	7.49±0.28
Dissolved Oxygen (mg l^{-1})	7.40±0.62	5.97±0.83	6.03±0.79	5.60±0.55
Nitrate (mg l^{-1})	0.01±0.007	0.01±0.004	0.03±0.013	0.01±0.004
Nitrite (mg l^{-1})	0.003±0.001	0.003±0.001	0.005±0.002	0.005±0.002
Ammonia (mg l^{-1})	0.07±0.02	0.15±0.05	0.17±0.04	0.23±0.07
Phosphate (mg l^{-1})	1.28±0.21	1.01±0.12	1.05±0.24	1.35±0.26
Chlorophyll-a (µg l^{-1})	200.1±40.9	230.4±49.1	182.1±39.6	189.5±25.7

Table 4. Plankton and benthic organism populations identified in Study 1. Values are mean abundance (×10^3 cells/L) ± SEM. No differences were observed between treatments. T1, Koi monoculture; T2-4, Koi-Rohu-Catla polyculture.

	T1 (100% feed)	*T2 (100% feed)*	*T3 (75% feed)*	*T4 (50% feed)*
Plankton				
Phytoplankton	365.38±19.65	181.04±18.00	276.75±24.88	236.38±10.26
Zooplankton	25.08±2.77	33.96±1.44	44.67±4.15	58.25±5.31
Total Plankton	390.46±17.62	215.00±16.64	321.42±22.64	294.63±9.39
Benthic Organisms				
Oligochaeta	651.9±162.4	425.9±92.8	385.2±86.5	272.8±41.7
Chironomid Larvae	433.3±87.0	586.4±141.8	446.91±113.9	269.1±29.1
Mollusca	242.0±49.3	203.7±28.8	293.8±59.9	232.1±54.8
Unidentified	9.88±2.11	12.35±3.07	25.9±7.76	9.88±3.33
Total Benthos	1337.0±276.2	1228.4±249.1	1151.9±200.7	783.9±113.9

Table 5. Growth and production parameters for Study 1. Values are mean ± SEM. Values with different letters are significantly different (Tukey's HSD; P < 0.05). NA = not applicable. T1, Koi monoculture; T2-4, Koi-Rohu-Catla polyculture.

	T1 (100% feed)	*T2 (100% feed)*	*T3 (75% feed)*	*T4 (50% feed)*
Koi (*A. testudineus*)				
Weight (g)	65.4±0.20^{a}	97.3±1.62^{ab}	120.4±7.82^{b}	121.5±1.32^{b}
Survival Rate (%)	60	47.5	55	57.35
Specific Growth Rate (% bw d^{-1})	3.79±0.002^{a}	4.12±0.014^{ab}	4.28±0.058^{b}	4.31±0.009^{b}
Gross Production (kg/ha)	1963.2±5.87^{a}	2311.6±38.4^{ab}	3311.0±215^{bc}	3484.0±37.8^{c}
Rohu (*L. rohita*)				
Weight (g)	NA	267.3±12.7^{a}	297.1±27.1^{a}	339.4±29.7^{a}
Survival Rate (%)	NA	61.25	58.33	60
Specific Growth Rate (% bw d^{-1})	NA	2.08±0.04^{a}	2.15±0.07^{a}	2.26±0.07^{a}
Gross Production (kg/ha)	NA	1309.5±62.1^{a}	1386.4±126.4^{a}	1629.1±142.4^{a}
Catla (*C. catla*)				
Weight (g)	NA	492.8±22.6^{a}	338.8±29.1^{b}	396.3±16.5^{ab}
Survival Rate (%)	NA	72.6	58	80
Specific Growth Rate (% bw d^{-1})	NA	2.26±0.037^{a}	1.93±0.073^{b}	2.08±0.033^{ab}
Gross Production (kg/ha)	NA	715.5±32.8^{a}	393.0±33.8^{b}	634.1±26.4^{a}
Total				
Feed Conversion Ratio	1.73±0.005^{a}	1.53±0.028^{ab}	1.31±0.077^{bc}	1.03±0.029^{c}
Gross Production (kg/ha)	1963.2±5.87^{a}	4336.7±77.9^{ab}	5090.4±248^{bc}	5747.2±164^{c}

Table 6. Economic analyses from Study 1. Values are mean ± SEM. Values with different letters are significantly different (ANOVA; P< 0.05). NA = not applicable. T1, Koi monoculture; T2-4, Koi-Rohu-Catla polyculture.

Financial Input *(BDT/ha)*	*T1 (100% feed)*	*T2 (100% feed)*	*T3 (75% feed)*	*T4 (50% feed)*
Bleaching Powder	5928	5928	5928	5928
Lime ($CaCO_3$)	10,338	10,338	10,338	10,338
Urea	593	593	7824	7824
Triple Super Phosphate	445	445	5975	5975
Koi	81,814	81,814	81,814	81,814
Rohu	0	37,400	37,400	37,400
Catla	0	15,584	15,584	15,584
Feed	168,207	317,290	315,632	283,550
Labor	10,000	10,000	10,000	10,000
Total Cost (BDT/ha)	277,325	479,392	490,495	458,413
Total Production (kg/ha)	1963.2±5.87^{a}	4336.7±77.9^{ab}	5090.4±248^{bc}	5747.2±164^{c}
Gross Return (BDT/ha)	353,376±1056^{a}	746,037±12670^{ab}	890,617±43361^{bc}	999,185±28185^{c}
Net Return (BDT/ha)	76,051±1056^{a}	266, 644±12670^{ab}	400,122±43361^{bc}	540,772±28185^{c}
Benefit Cost Ratio (BCR)	1.27	1.56	1.82	2.18

Table 7. Water quality parameters from Study 2. Values are mean ± SEM. Different letters indicate significant differences (Tukey's HSD; $P < 0.05$).

	T1 (0/m² Shing)	***T2 (1/m² Shing)***	***T3 (2/m² Shing)***	***T4 (3/m² Shing)***
Temperature (°C)	28.96 ± 0.037^a	29.04 ± 0.037^a	29.26 ± 0.161^a	29.31 ± 0.046^a
Transparency (cm)	28.71 ± 0.66^a	28.47 ± 0.66^a	24.05 ± 1.18^b	27.43 ± 0.59^{ab}
Alkalinity (mg l^{-1})	78.63 ± 0.67^a	81.11 ± 1.00^a	90.07 ± 1.07^b	87.11 ± 1.22^b
pH	7.38 ± 0.01^a	7.43 ± 0.02^a	7.56 ± 0.02^b	7.53 ± 0.01^b
Dissolved Oxygen (mg l^{-1})	6.95 ± 0.05^a	7.10 ± 0.04^{ab}	7.31 ± 0.07^b	6.96 ± 0.04^a
Nitrate (mg l^{-1})	0.12 ± 0.002^a	0.11 ± 0.007^a	0.14 ± 0.007^b	0.09 ± 0.008^a
Nitrite (mg l^{-1})	0.13 ± 0.005^a	0.15 ± 0.006^b	0.11 ± 0.001^a	0.16 ± 0.005^b
Ammonia (mg l^{-1})	0.22 ± 0.013^a	0.21 ± 0.005^a	0.17 ± 0.003^b	0.21 ± 0.003^a
Phosphate (mg l^{-1})	0.87 ± 0.007^a	0.91 ± 0.013^a	0.81 ± 0.020^a	0.84 ± 0.009^a
Chlorophyll-a (µg l^{-1})	135.07 ± 0.36^a	146.40 ± 0.53^b	155.12 ± 0.78^c	154.60 ± 0.71^c

Table 8. Plankton and benthic organism populations identified in Study 2. Values are mean abundance ($\times 10^3$ cells/L) ± SEM. Different letters indicate significant differences (Tukey's HSD; $P < 0.05$).

	T1 (0/m² Shing)	***T2 (1/m² Shing)***	***T3 (2/m² Shing)***	***T4 (3/m² Shing)***
Plankton				
Phytoplankton	480.52 ± 38.9^a	467.22 ± 6.35^a	543.67 ± 18.6^a	462.78 ± 13.6^a
Zooplankton	15.96 ± 0.39^a	15.85 ± 1.24^a	24.85 ± 2.29^b	19.44 ± 0.84^{ab}
Total Plankton	496.48 ± 38.8^a	483.07 ± 5.14^a	568.52 ± 16.8^a	482.22 ± 13.2^a
Benthic Organisms				
Oligochaeta	618.38 ± 6.11^a	530.59 ± 9.23^b	469.68 ± 15.5^c	369.82 ± 21.1^d
Chironomid Larvae	225.93 ± 12.4^a	433.33 ± 15.8^b	446.91 ± 2.39^c	586.42 ± 6.33^d
Mollusca	392.87 ± 2.90^a	370.37 ± 13.3^a	348.97 ± 37.5^a	305.62 ± 60.3^a
Unidentified	20.30 ± 0.55^a	22.50 ± 4.88^a	18.11 ± 2.51^a	19.75 ± 3.43^a
Total Benthos	1562.69 ± 8.08^a	1369.00 ± 10.5^{ab}	1198.35 ± 51.9^b	941.56 ± 85.7^c

Table 9. Growth performance outcomes for Study 2. Values are mean ± SEM. Values with different letters are significantly different (Tukey's HSD; $P < 0.05$). NA = not applicable.

	T1 (0/m² Shing)	***T2 (1/m² Shing)***	***T3 (2/m² Shing)***	***T4 (3/m² Shing)***
Koi (*A. testudinius*)				
Harvesting Weight (g)	103.96 ± 0.20^{a}	103.83 ± 0.26^{a}	107.29 ± 0.27^{b}	109.17 ± 0.36^{c}
Harvesting Length (cm)	14.33 ± 0.16^{a}	14.04 ± 0.11^{a}	14.25 ± 0.14^{a}	14.54 ± 0.18^{a}
Survival Rate (%)	62	70	67	65
Specific Growth Rate (% bw d^{-1})	2.327 ± 0.001^{a}	2.326 ± 0.002^{a}	2.349 ± 0.002^{b}	2.376 ± 0.007^{b}
Gross Production (kg/ha)	3222.71 ± 6.17^{a}	3634.17 ± 9.22^{b}	3594.27 ± 9.33^{c}	3547.92 ± 11.72^{d}
Rohu (*L. rohita*)				
Harvesting Weight (g)	152.42 ± 0.15^{a}	153.17 ± 0.19^{ab}	155.88 ± 0.16^{bc}	159.04 ± 0.53^{c}
Harvesting Length (cm)	24.67 ± 0.19^{a}	25.73 ± 0.16^{b}	25.54 ± 0.16^{b}	25.58 ± 0.15^{b}
Survival Rate (%)	87	89	93.5	82
Specific Growth Rate (% bw d^{-1})	1.385 ± 0.003^{a}	1.386 ± 0.001^{ab}	1.396 ± 0.003^{bc}	1.410 ± 0.004^{c}
Gross production (kg/ha)	1060.82 ± 1.04^{ab}	1090.55 ± 1.34^{bc}	1165.95 ± 1.23^{c}	1043.31 ± 3.48^{a}
Catla (*C. catla*)				
Harvesting Weight (g)	243.21 ± 0.30^{a}	246.08 ± 0.23^{b}	246.21 ± 0.24^{b}	246.5 ± 0.20^{b}
Harvesting Length (cm)	27.91 ± 0.12^{a}	28.42 ± 0.14^{a}	28.17 ± 0.16^{a}	28.44 ± 0.19^{a}
Survival Rate (%)	85	92	90	79
Specific Growth Rate (% bw d^{-1})	1.366 ± 0.002^{a}	1.376 ± 0.002^{b}	1.373 ± 0.001^{b}	1.374 ± 0.001^{b}
Gross Production (kg/ha)	413.45 ± 0.32^{ac}	452.79 ± 0.42^{b}	443.18 ± 0.44^{bc}	389.47 ± 0.32^{a}
Shing (*H. fossilis*)				
Harvesting Weight (g)	NA	104.38 ± 0.13^{a}	108.58 ± 0.20^{b}	106.96 ± 0.30^{c}
Harvesting Length (cm)	NA	21.94 ± 0.04^{a}	22.01 ± 0.09^{a}	21.98 ± 0.12^{a}
Survival Rate (%)	NA	68	72	67
Specific Growth Rate (% bw d^{-1})	NA	2.535 ± 0.001^{a}	2.563 ± 0.001^{b}	2.553 ± 0.002^{c}
Gross Production (kg/ha)	NA	709.75 ± 0.85^{a}	1563.60 ± 2.92^{b}	2149.86 ± 5.99^{c}
Total				
Feed Conversion Ratio	1.62 ± 0.051^{a}	1.59 ± 0.055^{a}	1.53 ± 0.035^{a}	1.86 ± 0.049^{b}
Gross Production (kg/ha)	4696.98 ± 6.85^{a}	5887.26 ± 10.37^{b}	6766.99 ± 9.17^{c}	7130.56 ± 14.43^{d}

Table 10. Economic analysis for Study 2. Values are mean ± SEM. Values with different letters are significantly different (Tukey's HSD; $P < 0.05$).

Financial Input *(BDT/ha)*	***T1 (0/m² Shing)***	***T2 (1/m² Shing)***	***T3 (2/m² Shing)***	***T4 (3/m² Shing)***
Bleaching Powder	5928	5928	5928	5928
Lime ($CaCO_3$)	10,338	10,338	10,338	10,338
Urea	7231	7231	7231	7231
Triple Super Phosphate	5530	5530	5530	5530
Koi	49,400	49,400	49,400	49,400
Rohu	55,328	55,328	55, 328	55,328
Catla	21,736	21,736	21,736	21,736
Shing	0	49,400	98,800	148,200
Feed	495,594	609,708	674,916	865,326
Labour	10,000	10,000	10,000	10,000
Total Cost (BDT/ha)	661,085	824,599	939,207	1,179,017
Total Production (kg/ha)	4696.98±6.85[a]	5887.26±10.37[b]	6766.99±9.17[c]	7130.56±14.43[d]
Gross Return (BDT/ha)	822,445±1224[a]	1,226,849±1959[ab]	1,615,276±1946[bc]	1,841,847±3505[c]
Net Return (BDT/ha)	161,360±1224[a]	402,250±1959[ab]	676,069±1946[c]	662,829±3505[bc]
Benefit Cost Ratio	1.24	1.49	1.72	1.56

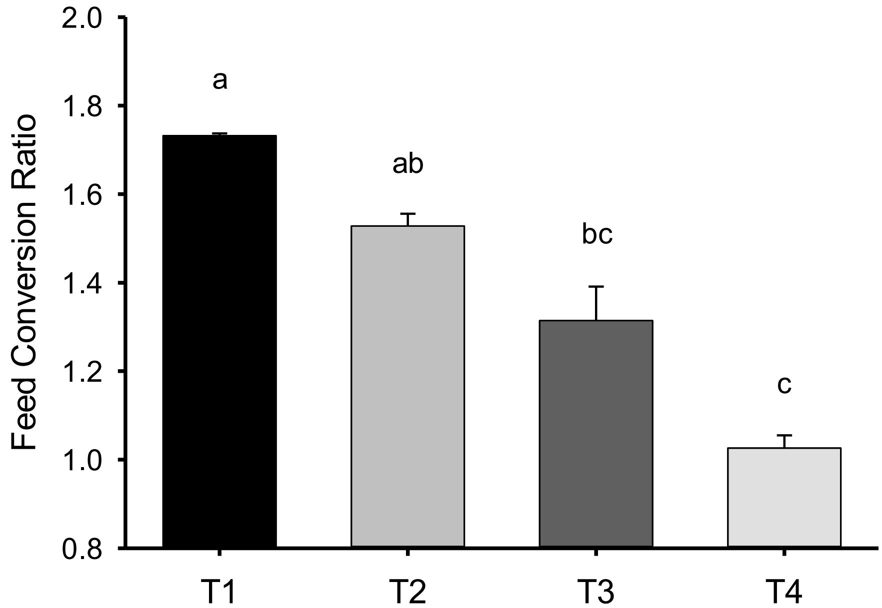

Figure 1. Feed conversion ratios for Koi monoculture (T1) and Koi-Carp polyculture (T2, 100% feed ration; T3, 75% feed ration; T4, 50% feed ration) in Study 1. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; $P < 0.05$).

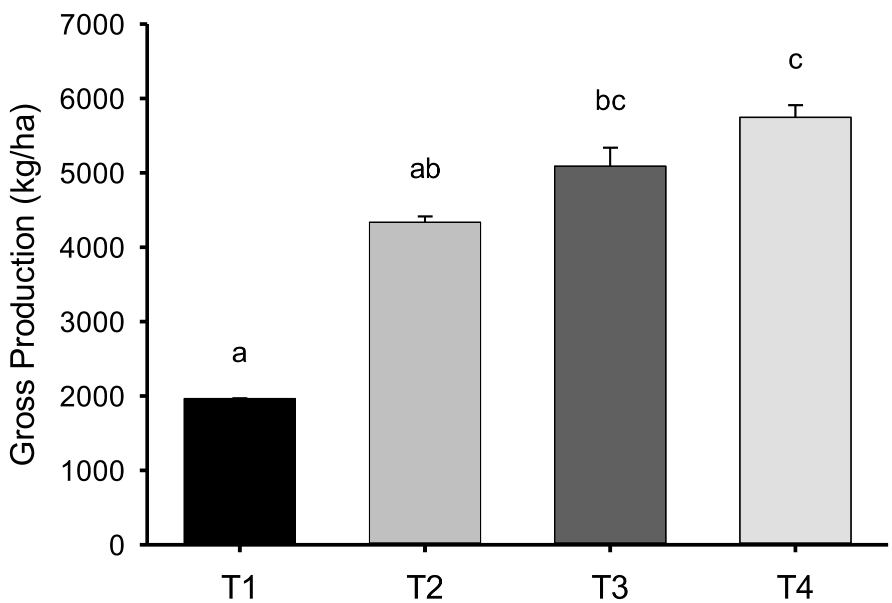

Figure 2. Gross production (kg/ha) for Koi monoculture (T1) and Koi-Carp polyculture (T2, 100% feed ration; T3, 75% feed ration; T4, 50% feed ration) in Study 1. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; $P < 0.05$).

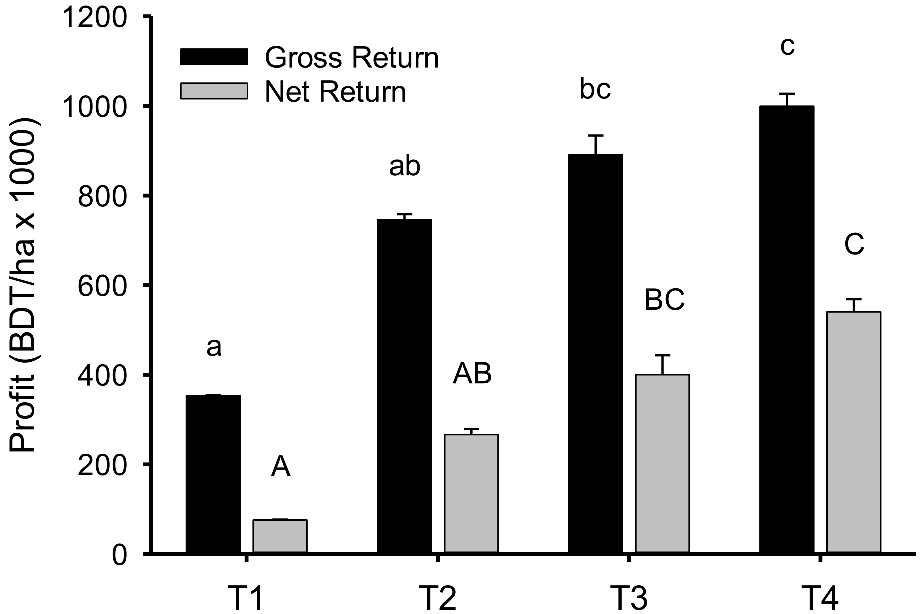

Figure 3. Gross (black) and net (grey) profits in Bangladesh Taka (BDT) for Koi monoculture (T1) and Koi-Carp polyculture (T2, 100% feed ration; T3, 75% feed ration; T4, 50% feed ration) in Study 1. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; $P < 0.05$).

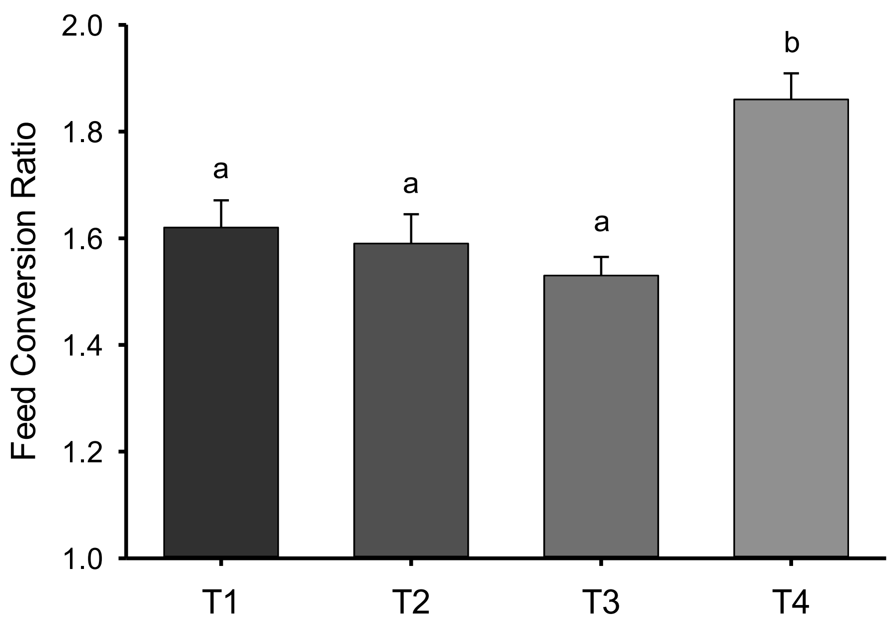

Figure 4. Feed conversion ratios for Koi-Carp (T1) and Koi-Carp-Shing (T2, 1/m^2 Shing; T3, 2/m^2 Shing; T4, 3/m^2 Shing) polyculture in Study 2. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; $P < 0.05$).

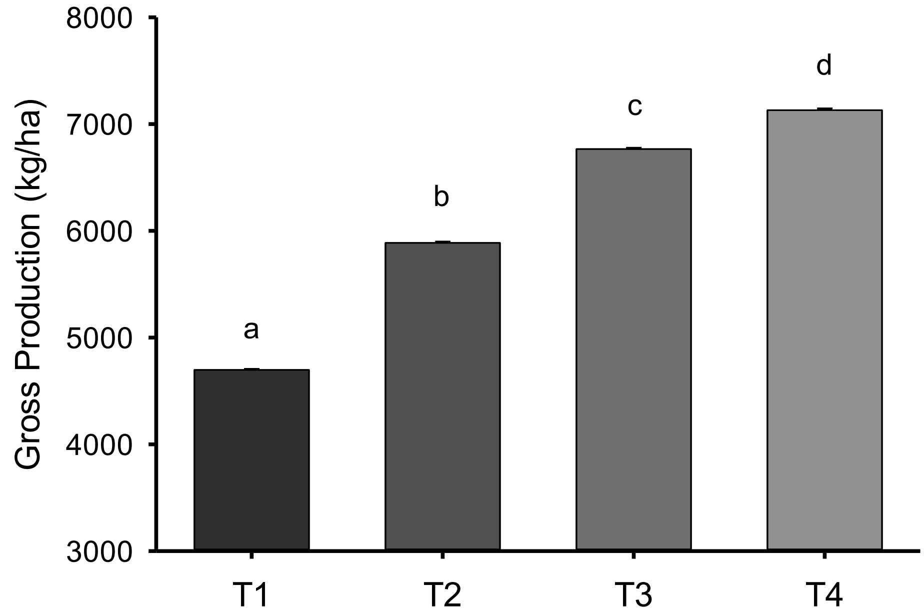

Figure 5. Gross production (kg/ha) for Koi-Carp (T1) and Koi-Carp-Shing (T2, 1/m^2 Shing; T3, 2/m^2 Shing; T4, 3/m^2 Shing) polyculture in Study 2. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; $P < 0.05$).

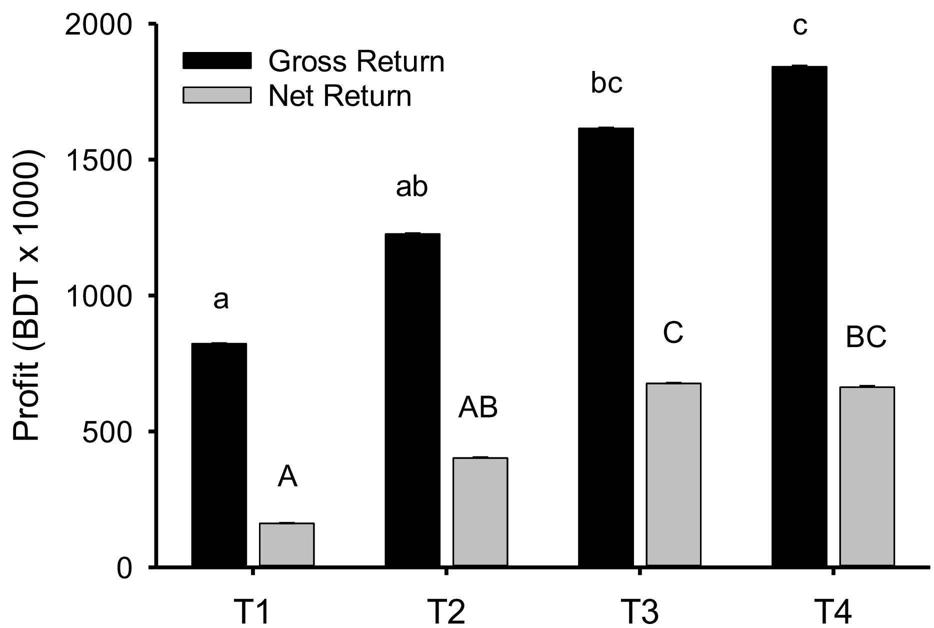

Figure 6. Gross (black) and net (grey) profits in Bangladesh Taka (BDT) for Koi-Carp (T1) and Koi-Carp-Shing (T2, 1/m^2 Shing; T3, 2/m^2 Shing; T4, 3/m^2 Shing) polyculture in Study 2. Values are mean ± SEM. Different letters indicate significant differences between treatments (Tukey's HSD; $P < 0.05$).