TOPIC AREA:
MITIGATING NEGATIVE ENVIRONMENTAL IMPACTS

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Novel Approach for the Semi-Intensive Polyculture of Indigenous Air-Breathing Fish With Carps for Increasing Income and Dietary Nutrition While Reducing Negative Environmental Impacts

Mitigating Negative Environmental Impacts/Experiment/13MNE01NC

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ABSTRACT

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. Currently, production of shing (*Heteropneustes fossilis*, stinging catfish) and koi (*Anabas testudineus,* climbing perch) is limited to monoculture systems with intensive use of commercial-grade feeds (30%–35% crude protein). As feed can comprise up to 60% of total production costs, the current practices for these fish limit participation by small homesteads and therefore comprise 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. Studies were carried out to determine: 1) if addition of Indian major carps (rohu, *Labeo rohita,* and catla, *Catla catla*) alone can provide cost benefits for growout of shing and if reducing feed by 50% from levels typically used by the industry to grow shing might provide further cost savings in shing-carp polyculture, and 2) what the effect of carp stocking density is on koi production using the best strategy established for shing-carp polyculture. The studies were carried out for 165 days in ponds at Bangladesh Agricultural University. The first experiment consisted of four treatments (T1, T2, T3, and T4) with four replications each. The stocking density was 5 shing/m^2, 0.8 rohu/ m^2 and 0.2 catla/m^2. All three fish species were stocked in all ponds except in the T1 group, representing the monoculture of shing as control. Ponds under T1 and T2 were fed at rates typically used in shing culture (20%– 5% body weight/day), while T3 received feed at 75% and T4 at 50% of full ration daily. Ponds were fertilized weekly in T3 and T4. Growth and production of fishes did not vary significantly in the different treatments. The survival rate of fish did not differ and ranged from 47% to 53% for shing and > 89% for carps. The net productions of fish in T1, T2, T3 and T4 were 623±155, 3069±774, 3280±853 and 3171±805 kg/ha, respectively. Net return was -103,827, 294,485, 442,711 and 542,215 BDT/ha and benefit cost ratio (BCR, total returns/total costs) was 0.82, 1.55, 1.93 and 2.37 in T1, T2, T3 and T4, respectively. T4 showed the best overall feed conversion ratio (FCR), BCR and fish yield followed by T3. The results show that feed and production efficiency is best in shing-carp polyculture under the 50% reduced feeding regimen.

Study 2 consisted of three treatments (T1, T2, and T3). Koi were stocked at the same density in all groups (5/m^2) and feed was applied at a 50% rate based on koi biomass. T1 was stocked with 0.8 rohu/m^2 and 0.2 catla/m^2, T2 with 1.0 rohu/m^2, and T3 with 1.0 catla/m^2. All ponds were fertilized weekly. There was no difference in weight gain or specific growth rate for any species between treatments or for survival rate for koi. The survival rate for rohu in T1 was significantly higher than T2 and for catla in T1 than in T3. Both gross and net production parameters were significantly higher for koi in T3 than in T1 or T2. Catla grown at a stocking rate of 1.0 fish/m^2 (T3) resulted in production parameters that were significantly higher than when stocked at 0.2 fish/m^2 (T1). There was no significant difference in production of rohu between treatments. There was no significant difference in FCR or BCR between treatments. In conclusion, the cost effectiveness of shing growout can be substantially improved by addition of carps to pond culture systems. Moreover, reducing feed inputs by half provides additional benefits to shing-carp polyculture with a 85% increase on returns. Based on these studies, farmers have the potential to enhance their income opportunities by incorporating carps and reducing feed inputs in the growout of shing catfish. Our studies also suggest that koi grow well with carps and production may be best with catla alone. Future studies are required to directly compare koi monoculture and koi-carp polyculture and the impacts of feed and fertilization inputs.

INTRODUCTION

Carps are the dominant finfish cultivated in Bangladesh, with multiple species farmed together in polyculture. Studies suggest that cultivation of other finfish varieties, particularly indigenous species with high mineral content, will be important steps 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). Indigenous air-breathing fishes, such as shing catfish (*Heteropneustes fossilis*) and koi (climbing perch, *Anabas testudineus*) have been successfully cultivated in Bangladesh in recent years and command a high market value (DOF 2012, Kohinoor et al. 2011). 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). This investigation seeks to promote production of finfish with high nutritional value (shing catfish and koi), while improving both economic profitability and environmental water quality through implementation of better management practices.

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 and drought. Currently, production of shing and koi is limited to monoculture systems with intensive use of commercial-grade feeds (30%–35% crude protein). As feed can comprise up to 60% of total production costs, the current practices for these fish limit participation by small homesteads and therefore comprise 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 (Wahab and Borski) observed firsthand that most air-breathing fish farms are often overfed, therefore 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 be mitigated through polyculture, where excess nutrients and algae can be utilized by other species (e.g., carp). This investigation will evaluate whether carps can be incorporated into the culture of indigenous air-breathing fishes, shing catfish and koi. As carps feed primarily upon primary production (phytoplankton/algae, Wahab et al. 2002), their incorporation may significantly reduce negative impacts associated with the farming of air-breathing fishes, while also allowing for greater production yields and the availability of additional fish for home consumption.

An additional mechanism for mitigating excess nutrient inputs is to limit the amount of feed applied in shing and koi culture. We have previously shown that equivalent production yields of tilapia can be achieved with 50% less feed either provided as a full ration on alternate days or as a lower daily rate (Bolivar et al. 2006, Borski et al. 2011). Similarly, alternate day feeding significantly improves feed conversion and reduces costs in the grow-out of milkfish in ponds and seacages (DeJesus-Ayson and Borski 2012). Feed-restriction has not been evaluated in shing catfish or koi production, however previous work in catfish (US and Asian varieties) suggests shing may also undergo periods of compensatory growth (SRAC 1989, Zhu et al. 2005). In particular, sutchi catfish (*Pangasianodon hypophthalmus*) raised on alternate-day feed regimens (50% feed reduction) had little differences in production yield compared to fish fed daily, yet net profits were increased 99% through the reductions in feed and labor costs (Amin et al. 2012). This investigation evaluated whether reduced-feeding protocols can be successfully applied to the polyculture of shing catfish/carp or koi/carp. Reductions in feed and overhead costs, combined with mixed-trophic level nutrient utilization, may make semi-intensive culture of shing catfish and koi more feasible for greater adoption among farmers while also mitigating environmental impacts associated with nutrient loading.

OBJECTIVES

- Assess reduced-feeding strategies for combined polyculture of two major carps (rohu, *Labeo rohita,* and catla, *Catla catla*) with shing catfish or koi in semi-intensive pond culture. Culture of carp with these fishes would represent a new polyculture technology in Bangladesh.
- Identify the feed-reduction strategy and carp stocking ratios needed for equivalent or better production yields through increased nutrient utilization efficiency.
- Evaluate overall performance and economic returns of the improved management strategy and transfer of findings to local farmers through an extension workshop.

MATERIALS AND METHODS

Study 1 — Assess reduced-feeding strategies for combined polyculture of two major carps (rohu and catla) with shing catfish. An experiment was carried out in 2013 prior to the start of this project to assess the semi-intensive polyculture of indigenous air-breathing fish shing catfish, with carps as a potential method for increasing income and reducing negative environmental impacts of shing catfish monoculture. rohu, catla and shing were stocked (rohu, 0.8 fish/m^2 ; catla, 0.2 fish/m^2 ; shing, 5 fish/m^2) in 9–100 m^2 ponds (1.5 m depth) in a completely randomized design allocated to three treatments: full daily feeding (T1), half daily feeding ration (T2), and alternative day full daily feeding (T3) under weekly fertilization rate for all treatments (N:P = 4:1). The combined net production for the three species was 3,300 kg/ha for T1, 2,136 kg/ha for T2, and 2,440 kg/ha for T3. Despite the higher net production of fish in T1, the benefit-to-cost ratio (returns ÷ investment) was better for T2 (3.34) and T3 (2.97) than for T1 (2.55). This is largely due to the lower costs of feed associated with 50% feed reduction strategies. Thus, despite lower production levels, daily feeding at half ration levels was the most cost effective strategy. The above novel approach demonstrates that carp can be polycultured with shing through utilization of natural food organisms along with reduced feed inputsedThis work was presented at the Aquaculture America meeting in February 2014 (Wahab et al. 2014).

Additional studies are required to directly evaluate shing-carp polyculture versus the current practice of shing monoculture to determine if the former allows for greater income generation for Bangladeshi farmers. Based on results from the first study, a revised research protocol was implemented. We eliminated alternate day feeding (benefit-cost ratio is less than that of 50% daily feeding) and replaced it with a 75% daily ration group that may result in growth that better approximates that observed under standard 100% feed ration and that yields improved growth over the 50% daily ration group, while still providing additional benefits of reduced feed inputs and the culture of Indian carps. The experimental design for this study is shown in Table 1.

This design contrasts the current practice of intensive shing catfish monoculture (T1) against treatments incorporating carps (T2), or semi-intensive culture with 75% and 50% reductions in daily ration (T3, T4). The treatment groups were randomly assigned to ponds (N =15, 100 m^2, 1.5 m depth). Prior to flooding and stocking, the ponds were dried, re-excavated, and limed (25 g $CaCO_3/m^2$). They were fertilized initially at 28 kg N and 5.6 kg P/ha prior to stocking. During the production period (165 days), T3 and T4 ponds were fertilized at a rate of 28 kg N/ha/week and 5.6 kg P/ha/week. Full rations of floating commercial feed (30% crude protein) was provided using a standard feeding rate currently employed by farmers (20% bw/day, 0–30 days; 15%, 31–60 days; 10%, 61–90 days; 5%, > 90 days). The reduced-feeding groups received either 75% or 50% less feed. Feed amounts were recorded for feed conversion and cost-benefit analysis performed at the end of study. All ponds were sub-sampled every 15 days for collection of weight and length. Rohu and catla were captured by seine net and bottom dwelling shing by cylindrical bamboo traps.

Fingerlings of rohu (~20 g), catla (~25 g) were collected from a local supplier and shing fingerlings (~2 g) from Authentic Matshya Hatchery, Mymensingh, Bangladesh. Fifty fingerlings of rohu, 20 fingerlings of catla and 50 fingerlings of shing were randomly sampled for measuring of length and weight prior to stocking.

Water quality was monitored daily (dO_2, pH, transparency by Secchi -disk depth), while additional parameters*,* e.g., ammonia, nitrites, nitrates, total phosphate, total alkalinity, and chlorophyll *a* were measured fortnightly by the Water Quality and Pond Dynamics Laboratory at BAU. Production yields (market weight, kg; length, g), estimated market returns, feed input costs (feed, fertilizers, fingerlings), and labor costs were gathered for all treatment groups at the end of study for marginal cost-benefit analysis.

The following equations were used to determine the growth parameters:

Weight gain (g) = Mean final weight − Mean initial weight

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

Net production = Number of fish harvested x Weight gain of fish

All treatments were tested for significant differences in growth (mean length, weight X time), growth efficiency (specific growth rate, feed conversion ratio), and water quality using Analysis of Variance ($p < 0.05$).

Study 2 — Effect of stocking density for koi/carp polyculture using the feeding-fertilization strategy developed for shing catfish. The 50% reduced ration with pond fertilization was identified to yield the best returns for shing catfish (T4, Study 1). We tested whether this management could be implemented for polyculture production of air-breathing koi with Indian carps (rohu and catla). As production of only one carp may prove useful under reduced-feeding, we also tested different stocking levels for these two carps. The experimental design used is shown in Table 2.

The treatment groups were randomly assigned to ponds (N = 12, 100 m^2, 1.5 m depth).
All treatment groups were fed a commercial diet (30% crude protein) at half the ration typically used by farmers (e.g. 20%–5% bw/day). Therefore, koi were fed at the beginning of the trial at 10% bw/day for 30 days, 7.5% bw/day for days 31–60, 5% for days 61–90, and 2.5% thereafter. Only the biomass of koi was

used for calculating feed inputs. The preparation of ponds, fertilization rates, and sample collection (growth data, water quality parameters) were performed as described in Study 1. As outlined in Study 1, the final production yields (market weight, kg; length, g), estimated market return, feed and labor costs were determined at the end of the study for an additional cost-benefit analysis. Treatments were tested for significant differences in growth (mean length, weight X time), growth efficiency (specific growth rate, feed conversion ratio), and water quality using analysis of variance (ANOVA) ($p < 0.5$).

The marginal cost-benefit for Experiments 1 and 2 (BAU) were addressed to determine whether semi-intensive polyculture of shing catfish and koi farming with Indian carps is economically more profitable and can reduce costs so smaller scale farmholders might adopt the practice. Water quality analyses determined the potential environmental benefits of the new semi-intensive polyculture technology on shing/koi culture. Results were presented to representatives from local extension agencies for further consideration and promotion to rural farmers. Perceived benefits from this analysis, including increased fish production, greater cost savings, market profitability, and feasibility for semi-intensive culture, along with promoting greater consumption of fish with high nutrient content (e.g., shing and koi), were extended to rural farmers through presentations at a local farmer's day event. The research outcomes were also disseminated through production of an extension factsheet in the local language for wider outreach to farmers, extension agencies of the government, and nongovernmental organizations (NGOs).

RESULTS AND DISCUSSION

Study 1 — Assess reduced-feeding strategies for combined polyculture of two major carps (rohu and catla) with shing catfish. This study evaluated the potential benefits of polyculture of shing with Indian major carps and of reduced feeding strategies on fish production. Water quality parameters are listed in Table 3. There were no differences in water quality among any of the treatments except for ammonia and nitrates, which were higher in the two treatments utilizing fertilizer to boost primary production of plankton (T3 and T4).

Four different groups of organisms were identified in the benthic samples collected in this study (Table 4). These included Chirnomid larvae, Oligocheta, mollusks, and unidentified organisms. The number of total benthic organisms were highest in treatments 3 and 4 (ponds that were fertilized weekly).

During the study period, five groups of phytoplankton were identified: Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae, and Rhodophyceae. In addition, four groups of zooplankton were identified in the experimental ponds: Cladocera, Copepoda, Rotifera, and Crustacea (Table 5). The total phytoplankton counts were highest in treatment 1 and total zooplankton counts were highest in treatment 3.

Table 6 summarizes the growth and production performance for Study 1. There was no difference in growth (weight and length) between any of the treatments for each species, shing catfish (Figure 1), rohu (Figure 2), or catla (Figure 3). There was also no difference in specific growth rate (SGR) or survival between the treatments (summarized in Table 6). Kohinoor et al. (2012) reported a harvesting weight of 49.50 g ± 4.52 g to 69.42 g ± 6.20 g and survival rate as 71% ± 2.64% to 87% ± 3.6% in a six month study of shing catfish monoculture. Chakraborty and Nur (2012) reported a mean harvesting weight of shing catfish varied between 30.24 g ± 3.91 g to 50.14 g ± 3.22 g in monoculture or in polyculture with koi (*A. testudineus*) and a survival rate of 64.70% ± 4.88% to 87.55% ± 2.02%. Stocking densities in these intensive culture systems ranged from 7.5 to 25 fish/m^2. The lower harvesting weight observed here is likely attributed to the spawning of the shing, which coincided with the slower rate of growth that occurred at around 90 days into the study.

There was no difference in gross or net production between treatments within species (Table 6). Total net fish production was higher in the polyculture treatments 2, 3, or 4 than in shing monoculture. Production values for carp polyculture of between 1,800 and 2,000 kg/ha have been previously reported (Mazid and Hossain 1999, Sharma and Thakur 1998) which are lower than those reported in this study (2,450–2,680 kg/ha). The feed conversion ratio (FCR) calculated for shing in treatment 1 was quite high due in part to survival rates of 50% and wastage of feed, exacerbated by lack of growth and reproductive activity found in the later 60 days of the study. It is possible that fish may have crawled and escaped from the ponds, particularly with heavy rains. Future studies should incorporate a barrier around the pond to prevent this potential occurance. Additionally, harvest of shing at an earlier period (100–120 days) when body weights tended to peak may have benefit both to reducing the FCR and improving returns on investment.

When we considered the economic return among treatment groups with a decreasing level of investment from treatment 1 to 4, this study found that the gross return for treatment 1 was significantly lower (negative) when compared to the other three treatments (Table 7). The highest economic returns were from the lowest feed input treatment groups (Figure 4). Benefit cost ratios were also highest in shing fed at 75% and 50% reduced ration in treatment 3 (BCR = 1.93) and 4 (BCR = 2.37), respectively. Moreover, a net positive BCR of 1.55 was seen when carps alone were introduce to shing culture

This study indicates that reduced feed rationing along with pond fertilization does not affect the growth or production of shing catfish, rohu, or catla in polyculture. Rohu and catla primarily feed on planktonic organisms, therefore application of feed does not contribute significantly to their growth, hence they can be supplemented with shing production systems without additional costly feed inputs. Taken together, these studies suggest: 1) that shing can be polycultured with major Indian carps with little impact on survival or growth of the species, 2) addition of carps to shing culture increases the net production of fish and provides greater returns on investment that shing monoculture, and 3) combined pond fertilization and application of 50% less feed further improves economic returns by > 50% of shing-carp polyculture with no impact on fish production.

Study 2 — Effect of stocking density for koi/carp polyculture using the feeding-fertilization strategy developed for shing catfish. This study was designed to assess to determine whether air-breathing koi (Study 1) can be polycultured with Indian carps (rohu and catla). We also tested if there might be benefits to koi-carp polyculture when stocking rohu or catla alone or both in at a 4:1 rohu:catla ratio. Based on the feeding protocol that yielded the best results in shing-carp polyculture in Study 1, fish were fed at half the recommended ration (e.g. 20%–2.5% bw/day). Application of feed was based only on biomass of koi. All ponds were fertilized to provide primary productivity for fishes, namely carps. We found no difference in water quality among any of the treatments assessed in this study (Table 8).

Four different groups of organisms were identified in the benthic samples collected in this study (Table 9). These included Chirnomid larvae, Oligocheta, mollusks, and unidentified organisms. The number of total benthic organisms were highest in treatment 3 (koi raised with catla alone).

During the study period, six groups of phytoplankton were identified: Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae, Xanthophyceae, and Rhodophyceae. In addition, five groups of zooplankton were identified: Cladocera, Copepoda, Rotifera, Crustacea, and Protozoa were identified in the experimental ponds (Table 10). Although total phytoplankton counts were highest in treatment 2 and total zooplankton counts were highest in Treatment 3, there were no significant difference in plankton counts among the treatment groups.

Table 11 summarizes the growth and production performance for Study 2. There was no difference in growth (weight) between any of the treatments for each species, koi, rohu, or catla. When cage cultured, koi raised at 150 fish/m^3 had the greatest mean harvesting weight of 118.60 g ± 2.535 g (Habib et al. 2015). Koi harvesting weights have been reported between 86.02 g ± 1.02 g to 90.00 g ± 2.00 g (Helal

2014). Mean harvesting weight in the present study was substantially higher (129–148 g; Figure 5, Table 11) which may relate to differences in feed, fertilizers, and stocking density used among the studies.

The survival rate of koi did not vary significantly between treatment (64.36% ± 9.92% to 77.36% ± 11.74%; Figure 6; Table 11). Ahmed et al. (2015) reported the highest survivability of koi was 87% when stocked at a rate of 150 fry/dec (3.75 fry/m^2) but was 69% when stocked at a rate of 350 fry/dec (8.75 fry/m^2). Here, ponds were stocked at a rate of 5 koi/m^2, and survival rates fell within the range observed in these other studies. Catla and rohu survival declined when each was cultured alone compared to when they were cultured together with koi ($p < 0.01$; Figure 6). Variation in stocking density may affect survival rates and final production of carps, although there were no significant differences in specific growth rate for each species among the treatments (Figure 6).

Gross and net production of koi was significantly higher when koi were grown with catla (treatment 3) than with rohu only (treatment 2) or with both catla and rohu (Treatment 1; Table 11, Figure 7). Both parameters were greater for catla when the fish was grown alone at 1.0/m^2 with koi that when grown at a stocking density of 0.2/m^2 with rohu and koi. No differences in gross or net production was found with rohu whether cultured alone with koi or in combination with catla and koi. The combined gross production in this study did not vary between treatments but the combined net production was highest in treatment 3 (the combined culture of koi and catla) than in treatment 2 (the combined culture of koi and rohu, Figure 10d). The overall feed conversion ratio (FCR) based on biomass of all fishes did not significantly vary between treatment (Figure 8). The benefit cost ratio (BCR) was highest for treatment 1 (1.95) followed by treatment 3 (1.87) and treatment 2 (1.86). However, there was no significant difference in BCR between any treatment (Table 12).

Taken together, these results indicate that koi can be polycultured with carps and that this system produces significant returns on investment of around 450,000 BDT/ha when fish are fed at a rate of 10% bw/day–2.5% bw/day and ponds are fertilized weekly. While the best polyculture production may occur when koi are solely cultured with catla, koi can also be cultured with either rohu alone or both rohu and catla at a 4:1 ratio.

CONCLUSION

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, poor water quality and other conditions. Currently, production of the air breathing fishes, shing and koi are limited to monoculture systems with intensive use of commercial-grade feeds (30%–35% crude protein) at rates of application from 20% down to 5% body weight/day. As feed can comprise up > 80 % of total production costs, as demonstrated here, the current practices for these fish limit participation by small homesteads and therefore comprise a significant impediment to further expansion of this industry. The research presented here suggests that addition of major Indian carps (rohu and catla) to shing catfish enhances total fish yields, increases return on investment with little impact on growth of shing. Reducing the daily rate of feeding by 50% provides an additional 80% profit. Farmers, therefore, have the potential to enhance their income opportunities by incorporating carps and reducing feed inputs in the growout of shing catfish while providing a more sustainable product. Our studies also suggest that koi grow well with carps and production may be best with catla alone. Future studies are required to directly compare koi monoculture and koi-carp polyculture and the impacts of feed and fertilization inputs. Two extension brochures were produced and a farmers day workshop was provided to disseminate the new polyculture technologies. Several M.S. graduate students also received research training to build capacity in the Bangladesh fisheries and aqaculture sectors.

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TABLES AND FIGURES

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)
Shing (*H. fossilis*)	200 (5.0/ m^2)	200 (5.0/ m^2)	200 (5.0/ m^2)	200 (5.0/ m^2)
Fertilization	0	0	4:1 (N: P)	4:1 (N: P)
Feeding Protocol	100% ration	100% ration	75% ration	50% ration
Replicates (*n*)	3	4	4	4

Table 2. Experimental design for Study 2.

	Treatment 1	***1.1.1*** *Treatment 2*	*Treatment 3*
Rohu (*L. rohita*)	80 (0.8/m^2)	100 (1.0/m^2)	none
Catla (*C. Catla*)	20 (0.2/m^2)	none	100 (1.0/m^2)
Koi (*A. testudineus*)	500 (5.0/m^2)	500 (5.0/m^2)	500 (5.0/m^2)
Fertilization/Feeding	50% daily ration	50% daily ration	50% daily ration
Replicates (*n*)	4	4	4

Table 3. Water quality parameters from Study 1. Values are mean ± SD. Values with different letters are significantly different ($P < 0.05$).

	Treatment 1	*Treatment 2*	*Treatment 3*	*Treatment 4*
Temperature (°C)	30.31±1.59	30.33±1.43	30.18±1.42	30.26±1.44
Transparency (cm)	24.42±14.44	25.31±11.33	25.43±10.90	27.10±10.62
Total Alkalinity (mg/L)	86.61±32.90	95.89±36.96	89.27±37.48	93.57±36.56

pH	7.49±0.46	7.54±0.49	7.51±0.48	7.53±0.50
Dissolved Oxygen (mg/L)	6.31±0.81	6.19±0.85	6.18±0.84	6.30±0.83
Nitrate (mg/L)	0.08±0.0[c]	0.08±0.07[c]	0.25±0.26[a]	0.16±0.17[b]
Nitrite (mg/L)	0.08±0.15	0.08±0.13	0.13±0.17	0.10±0.15
Ammonia (mg/L)	0.26±0.23[a]	0.17±0.14[a]	0.32±0.43[ab]	0.48±0.66[b]
Phosphate (mg/L)	0.68±0.43	0.75±0.49	0.82±0.54	0.66±0.48
Chlorophyll-a (mg/L)	323.67±266.18	243.54±216.44	249.64±223.71	194.63±36.56

Table 4. Benthic organisms identified in Study 1. Values are mean abundance ($\times 10^3$ cells/L) ± SD.

	Treatment 1	*Treatment 2*	*Treatment 3*	*Treatment 4*
Oligochaeta	125.43±90.07	113.33±46.91	137.77±37.75	90.37±28.39
Chironomidae	356.54±93.03	783.70±114.35	978.51±137.04	1057.03±287.55
Mollusca	61.23±31.07	48.88±23.47	40±15.05075	69.62±18.83
Unidentified	26.66±5.04	80±18.28	82.96±17.17	42.22±14.22
Total Benthos	569.87±172.95	1,025.92±140.03	1,239.25±134.91	1,259.25±294.45

Table 5. Plankton populations identified in Study 1. Values are mean abundance ($\times 10^3$ cells/L) ± SD.

	Treatment 1	*Treatment 2*	*Treatment 3*	*Treatment 4*
Chlorophyceae	71.27±4.67	71.25±3.03	80.72±4.14	82.90±3.89
Cyanophyceae	108.09±13.34	51.01±3.43	75.76± 8.08	57.92±4.44
Bacillariophyceae	27.90±1.77	27.90±1.64	31.58±1.87	30.90±1.65
Euglenophyceae	16.41±2.73	22.70±2.99	30.21±5.84	41.10±5.44
Rhodophyceae	3.53±0.64	4.63±0.69	4.98±0.75	5.16±0.66
Total phytoplankton	232.75±14.8	178.78±7.11	224.70±12.07	217.72±10.76
Rotifera	13.06±1.40	13±1.41	18.89±2.29	16.69±1.60
Crustacea	1.35±0.34	1.05±0.24	0.92±0.24	1.14±0.25
Cladocera	0.48±0.24	0.80±0.27	0.65±0.24	0.90±0.23
Copepoda	0.94±0.27	0.43±0.13	0.58±0.17	0.87±0.19
Total Zooplankton	16.07±1.79	16.00±1.59	21.38±2.24	20.21±1.91
Total Plankton	248.82±1.52	194.78±7.55	246.08±1.28	237.95±1.11

Table 6. Growth performance outcomes for Study 1. Values are mean ± SD. Values with different letters are significantly different ($P < 0.05$). NA = not applicable.

	Treatment 1	*Treatment 2*	*Treatment 3*	*Treatment 4*
Shing *(Heteropneustes fossilis*)				
Stocking Weight (g)	1.13 ± 0.98	1.13 ± 0.98	1.13 ± 0.98	1.13 ± 0.98
Harvesting Weight (g)	24.73±3.45	20.62±3.50	22.70±2.34	24.44±5.09
Weight Gain (g)	23.60±3.45	19.49±3.50	21.57±2.34	23.31±5.09
Initial Length (cm)	5.53±1.67	5.53±1.67	5.53±1.67	5.53±1.67
Final Length (cm)	16.08±2.10	15.28±2.12	15.59±2.18	15.99±2.77
Net Length (cm)	10.55±2.10	9.75±2.12	10.06±2.18	10.46±2.77
Survival Rate (%)	53.00±6.36	47.50±5.02	52.00±2.44	53.35±2.00
Specific Growth Rate, SGR (%)	1.83±0.08	1.72±0.10	1.78±0.06	1.82±0.13
Gross Production (kg/ha)	652.21±158.42	483.65±100.90	583.71±70.80	644.23±134.30
Net Production (kg/ha)	622.63±155.25	457.14±99.46	554.68±69.99	614.44±134.09
Rohu (*Labeo rohita*)				
Stocking Weight (g)	NA	21.76± 7.76	21.76± 7.76	21.76± 7.76
Harvesting Weight (g)	NA	304.81±106.6	309.63±103.26	279.92±119.29
Weight Gain (g)	NA	283.05±106.61	287.87±103.26	258.16±119.29
Initial Length (cm)	NA	12.48±1.40	12.48±1.40	12.48±1.40
Final Length (cm)	NA	28.95±2.54	29.58±2.08	28.02±3.34
Net Length (cm)	NA	16.47±2.54	17.1±2.08	15.54±3.34
Survival Rate (%)	NA	90.63±8.92	89.38±8.75	92.19±3.73
Specific Growth Rate, SGR (%)	NA	1.54±0.22	1.55±0.21	1.48±0.27
Gross Production (kg/ha)	NA	2126.67±561.52	2218.06±874.12	2062.45±941.87
Net Production (kg/ha)	NA	1970.80±575.10	2064.34±863.13	1903.90±936.21
Catla (*Catla catla*)				
Stocking Weight (g)	NA	27.30± 8.23	27.30 ± 8.23	27.30 ± 8.23
Harvesting Weight (g)	NA	368.60±117.88	402.08±57.89	389.40±119.54
Weight Gain (g)	NA	341.30±117.88	374.78±57.89	362.10±119.54
Initial Length (cm)	NA	13.05±1.36	13.05±1.36	13.05±1.36
Final Length (cm)	NA	29.63±2.24	30.46±1.58	29.69±3.48
Net Length (cm)	NA	16.58±2.24	17.41±1.58	16.64±3.48
Survival Rate (%)	NA	96.25±3.50	88.75±10.15	91.25±4.79
Specific Growth Rate, SGR (%)	NA	1.53±0.20	1.60±0.09	1.56±0.18
Gross Production (kg/ha)	NA	692.84±196.38	708.89±163.02	701.48±220.48
Net Production (kg/ha)	NA	640.91±197.96	661.01±157.20	652.26±220.21
Combined				
Feed Conversion Ratio, FCR	15.23±3.26^{b}	2.35±0.61^{a}	1.74±0.48^{a}	1.37±0.49^{a}
Gross Production (kg/ha)	652.21±158.42^{a}	3303.16±825.23^{b}	3510.66±904.85^{b}	3408.16±853.72^{b}
Net Production (kg/ha)	622.63±155.25^{a}	3068.85±774.45^{b}	3280.04±852.98^{b}	3170.60±805.46^{b}

Table 7. Economic analyses from Study 1. Values are mean ± SD. Values with different letters are significantly different ($P < 0.05$). NA = not applicable.

	Treatment 1	*Treatment 2*	*Treatment 3*	*Treatment 4*
Financial Input (BDT/ha)				
Lime	2,470	2,470	2,470	2,470
Urea	1,033[a]	1,033[a]	20,659[b]	20,659[b]
TSP	729[a]	729[a]	14,582[b]	14,582[b]
Shing	61,750	61,750	61,750	61,750
Rohu	NA	39,520	39,520	39,520
Catla	NA	19,760	19,760	19,760
Feed	517,003[d]	398,449[c]	309,733[b]	228,050[a]
Labor and Others	10,000	10,000	10,000	10,000
Total Cost	592,986[d]	533,712[b]	478,475[c]	396,792[a]
Financial Return (BDT/ha)				
Shing	489,159	362,738	437,783	483,169
Rohu	NA	361,534	377,070	350,617
Catla	NA	103,925	106,334	105,222
Total Return	489,159[a]	828,197[b]	921,187[b]	939,008[b]
Net Return	-103,827[a]	294,485[b]	442,711[b]	542,215[b]
BCR	0.82[a]	1.55[b]	1.93[bc]	2.37[c]

Note: Sale price of shing catfish, rohu and catla were 750, 170 and 150 BDT/kg, respectively.

Table 8. Water quality parameters from Study 2. Values are mean ± SD.

	Treatment 1	*Treatment 2*	*Treatment 3*
Temperature (°C)	29.39±1.25	29.57±1.22	29.60±1.45
Transparency (cm)	28.01±14.82	24.22±14.39	28.12±16.30
Total Alkalinity (mg/L)	136.33±39.83	141.59±34.48	135.96±36.32
pH	7.88±0.48	7.89±0.57	7.79±0.46
Dissolved Oxygen (mg/L)	5.22±1.43	4.93±1.43	5.04±1.60
Nitrate (mg/L)	0.19±0.18	0.20±0.19	0.22±0.18
Nitrite (mg/L)	0.13±0.15	0.10±0.15	0.13±0.13
Ammonia (mg/L)	0.34±0.26	0.35±0.57	0.31±0.33
Phosphate (mg/L)	1.51±0.81	1.14±0.83	1.20±0.70
Chlorophyll-a (µg/L)	96.01±95.32	108.93±102.06	158.90±158.01

Table 9. Benthic organisms identified in Study 2. Values are mean abundance ($\times 10^3$ cells/L) ± SD. Values with different letters are significantly different (P < 0.05).

	Treatment 1	*Treatment 2*	*Treatment 3*
Oligochaeta	230.45±237.63[a]	222.63±240.64[a]	436.63±487.81[b]
Chironomidae	590.53±494.35[a]	505.35±455.65[a]	1226.7±1156.41[b]
Mollusca	289.30±313.44	258.85±308.11	274.90±272.99
Unidentified	12.34±29.94	9.46±22.71	7.40±16.79
Total	1122.62±696.04[a]	996.29±646.25[a]	1945.63±1520.50[b]

Table 10. Plankton populations identified in Study 2. Values are mean abundance ($\times 10^3$ cells/L) ± SD. Values with different letters are significantly different (P < 0.05).

	Treatment 1	*Treatment 2*	*Treatment 3*
Bacillariophyceae	10.06±6.86	14.81±13.84	10.34±9.06
Chlorophyceae	14.50±15.50	12.47±11.61	10.09±9.70
Cyanophyceae	25.84±42.95	37.75±54.03	18.81±39.55
Euglenophyceae	2.81±4.05	2.75±3.68	4.88±11.88
Rhodophyceae	0.00±0.00	0.00±0.00	0.00±0.00
Xanthophyceae	0.00±0.00	0.00±0.00	0.03±0.18
Total phytoplankton	53.22±44.40	67.78±50.93	44.16±42.16
Copepoda	7.44±5.92[c]	12.41±10.30[a]	8.03±6.00[b]
Crustacea	0.00±0.00	0.00±0.00	0.00±0.00
Rotifera	17.56±21.80[b]	6.38±8.31[c]	20.66±23.67[a]
Cladocera	1.84±3.36[c]	7.25±15.18[a]	3.88±7.52[b]
Protozoa	0.00±0.00	0.00±0.00	0.00±0.00
Total zooplankton	26.84±24.10	26.03±23.95	32.56±26.39
Total plankton	80.06±44.72	93.81±54.40	76.72±43.81

Table 11. Growth performance outcomes for Study 1. Values are mean ± SD. Values with different letters are significantly different ($P < 0.05$). NA = not applicable.

	Treatment 1	*Treatment 2*	*Treatment 3*
Koi (*A.testudinius*)			
Stocking Weight (g)	2.94±0.87	2.94±0.87	2.94±0.87
Harvesting Weight (g)	129.24±36.27	148.39±28.39	144.13±15.79
Weight Gain (g)	126.30±36.27	145±28.39	141.19±15.79
Survival Rate (%)	72.64±23.46	64.36±9.92	77.36±11.74
Specific Growth Rate (SGR) (%/day)	3.13±0.25	3.26±0.17	3.24±.09
Gross Production (kg/ha)	4,324.76±390.17^{b}	4,617.63±374.3^{b}	5,459.23±532.17^{a}
Net Production (kg/ha)	4,219.25±378.42^{b}	4,524.16±381.24^{b}	5,346.88±521.64^{a}
Rohu (*L. rohita*)			
Stocking Weight (g)	22.92±3.20	22.92±3.20	NA
Harvesting Weight (g)	162.6±33.35	142.08±22.48	NA
Weight Gain (g)	139.68±33.35	119.16±22.48	NA
Survival Rate (%)	99.69±0.63^{a}	90.88±7^{b}	NA
Specific Growth Rate (SGR) (%/day)	1.62±0.17	1.51±0.13	NA
Gross Production (kg/ha)	1,282.11±268.31	1,272.06±188.51	NA
Net Production (kg/ha)	1,101.52±267.44	1,066.28±185.65	NA
Catla (*G. catla*)			
Stocking Weight (g)	30.7±10.29	NA	30.7±10.29
Harvesting Weight (g)	243.85±92.72	NA	198.7±44.10
Weight Gain (g)	213.15±92.72	NA	168±44.10
Survival Rate (%)	84.17±9.86^{a}	NA	54.13±7.92^{b}
Specific Growth Rate (SGR) (%/day)	1.68±0.34	NA	1.54±0.2
Gross Production (kg/ha)	394.57±116.59^{b}	NA	1,086.29±356.48^{a}
Net Production (kg/ha)	343.51±120.07^{b}	NA	922.12±333.45^{a}

Table 12. Economic analyses from Study 2. Mean values with different letters are significantly different ($P < 0.05$). NA = not applicable.

	Treatment 1	*Treatment 2*	*Treatment 3*
Financial Input (Taka/ha)			
Salt	5,928	5,928	5,928
Lime	8,892	8,892	8,892
Urea	7,231	7,231	7,231
TSP	5,529	5,529	5,529
Koi	98,800	98,800	98,800
Rohu	39,520[b]	49,400[a]	NA
Catla	17,784[b]	NA	88,920[a]
Feed	280,901	302,308	299,757
Labor and Others	10,000	10,000	10,000
Total Cost	474,585	488,088	525,057
Financial Return (Taka/ha)			
Koi	648,713[b]	692,645[b]	818,885[a]
Rohu	217,959	216,250	NA
Catla	59,186[b]	NA	162,943[a]
Total Return	925,858	908,895	981,828
Net Return	451,273	420,807	456,771
BCR (Benefit Cost Ratio)	1.95	1.86	1.87

Note: Sale price of koi, rohu and catla were 150, 170 and 150 BDT/kg, respectively

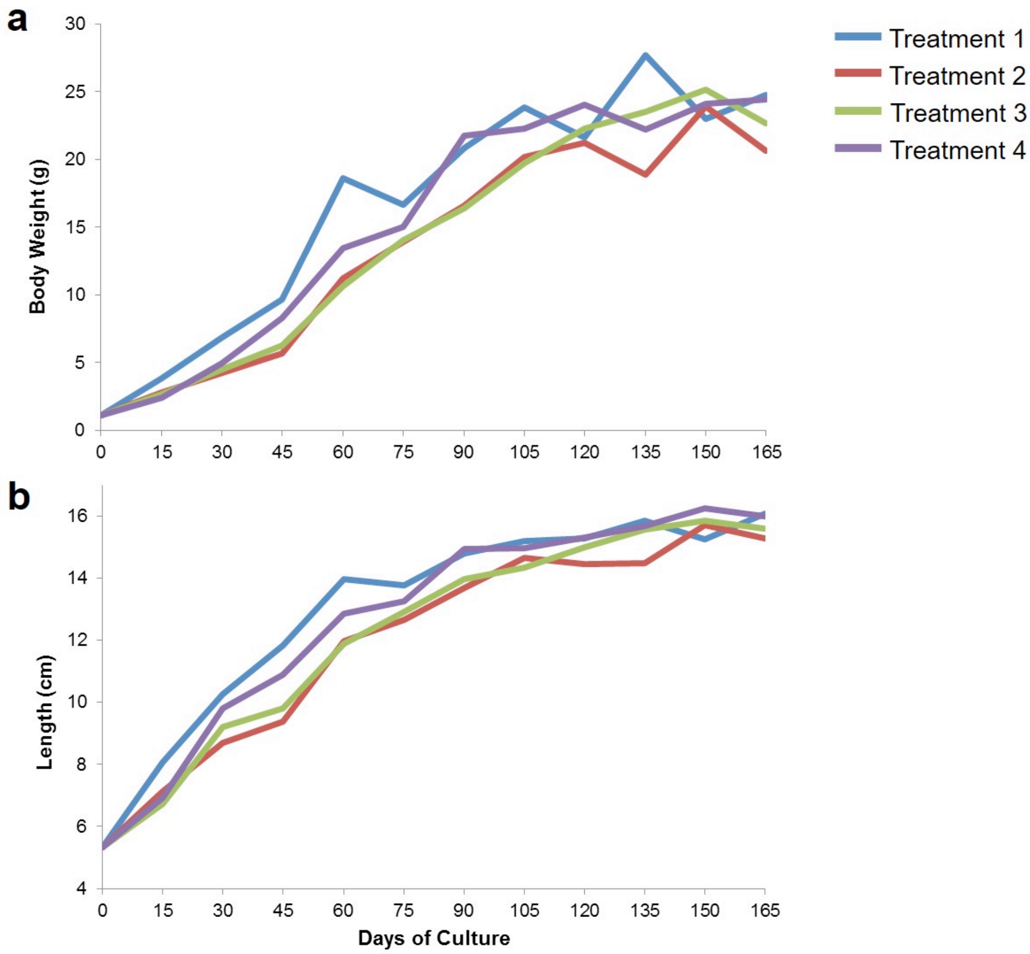

Figure 1. Weight (a) and length (b) of shing catfish (*H. fossilis*) at 15 day sampling periods in Study 1. There was no significant difference in weight or length between treatments throughout the study. Values are mean of the metric at the time of sampling.

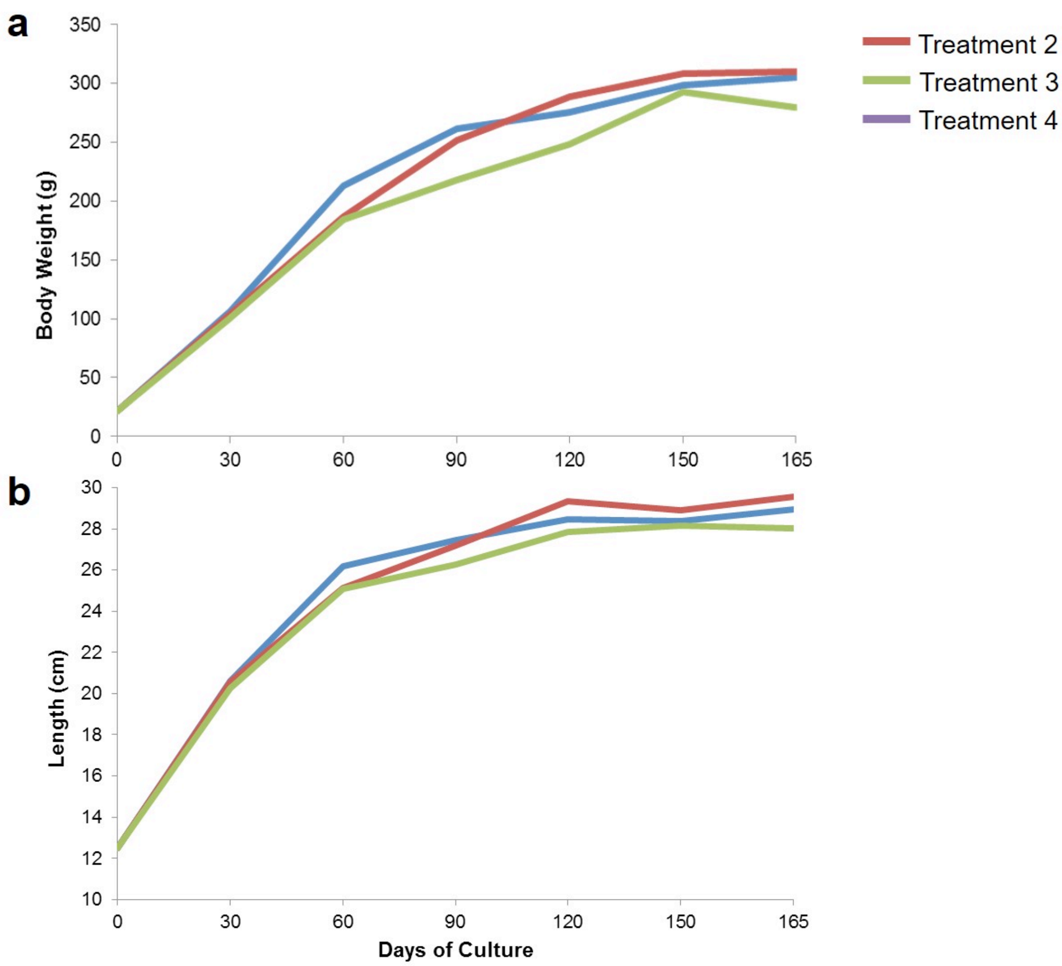

Figure 2. Weight (a) and length (b) of rohu (*L. rohita*) at 15 day sampling periods in Study 1. There was no significant difference in weight or length between treatments throughout the study. Values are mean of the metric at the time of sampling.

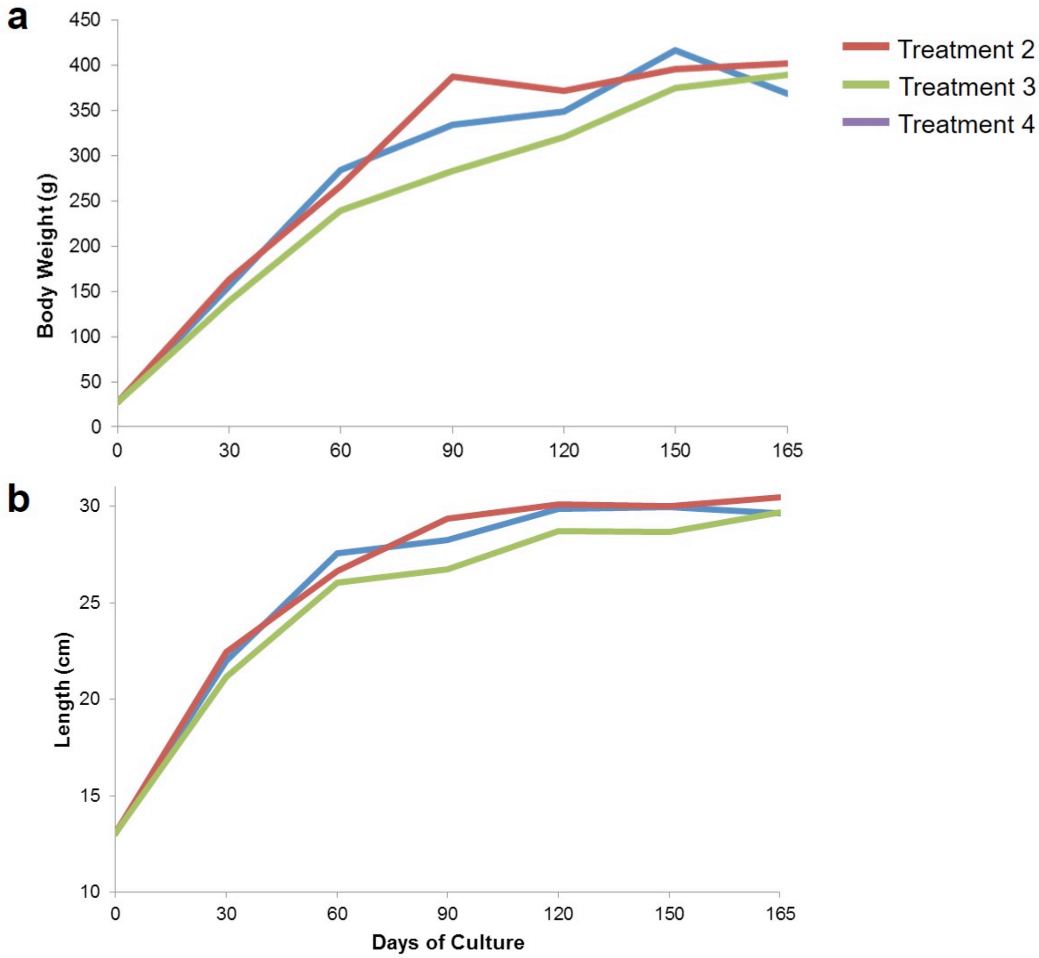

Figure 3. Weight (a) and length (b) of catla (*C. catla*) at 15 day sampling periods in Study 1. There was no significant difference in weight or length between treatments throughout the study. Values are mean of the metric at the time of sampling.

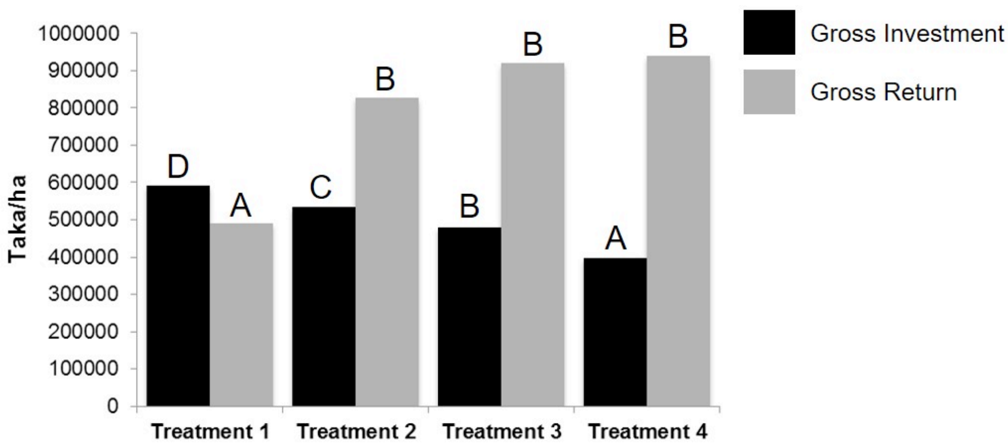

Figure 4. Combined gross investment and return for shing catfish (*H. fossilis*), rohu (*L. rohita*), and catla (*C. catla*) in Study 1. The gross investment was significantly lower in treatment 4 where shing catfish were raised along with both rohu and catla in fertilized ponds fed on alternate days. The gross return for treatment 1 was significantly lower than treatments 2, 3, and 4. Values are mean for treatments. Bars with different letters represent significant differences among treatments ($p < 0.05$).

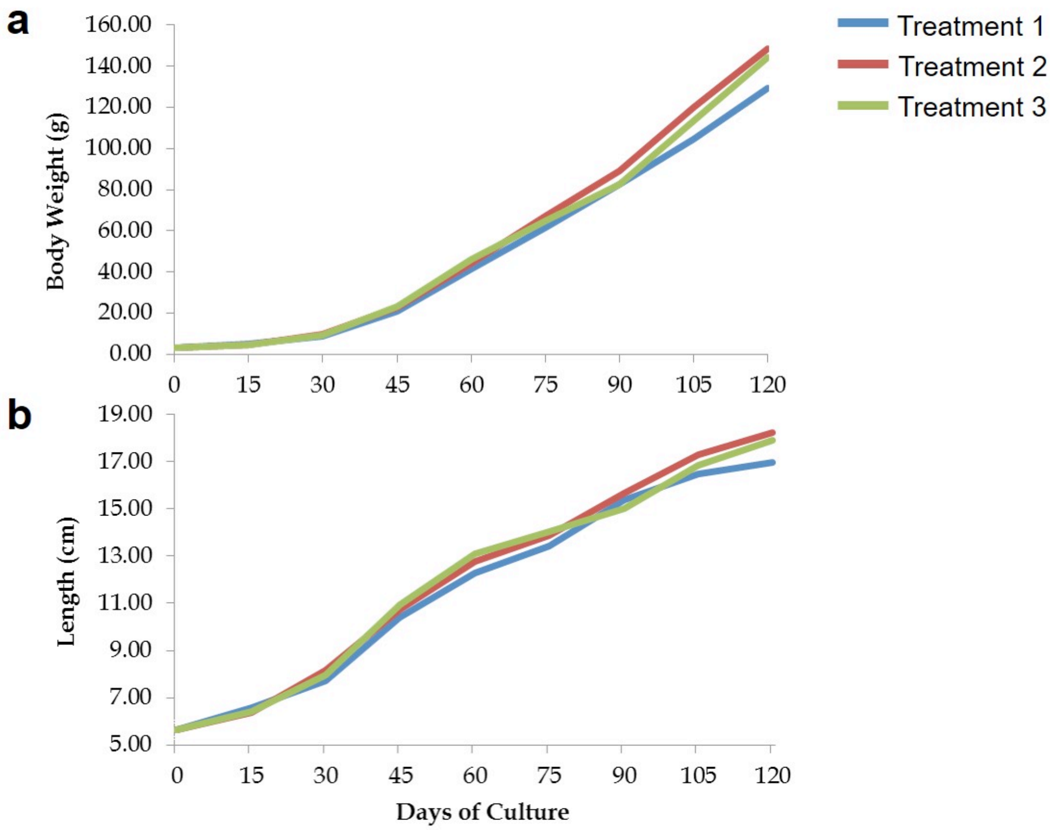

Figure 5. Weight (a) and length (b) of koi (*A. testudineus*) at 15 day sampling periods in Study 2. There was no significant difference in weight or length between treatments throughout the study. Values are mean of the metric at the time of sampling.

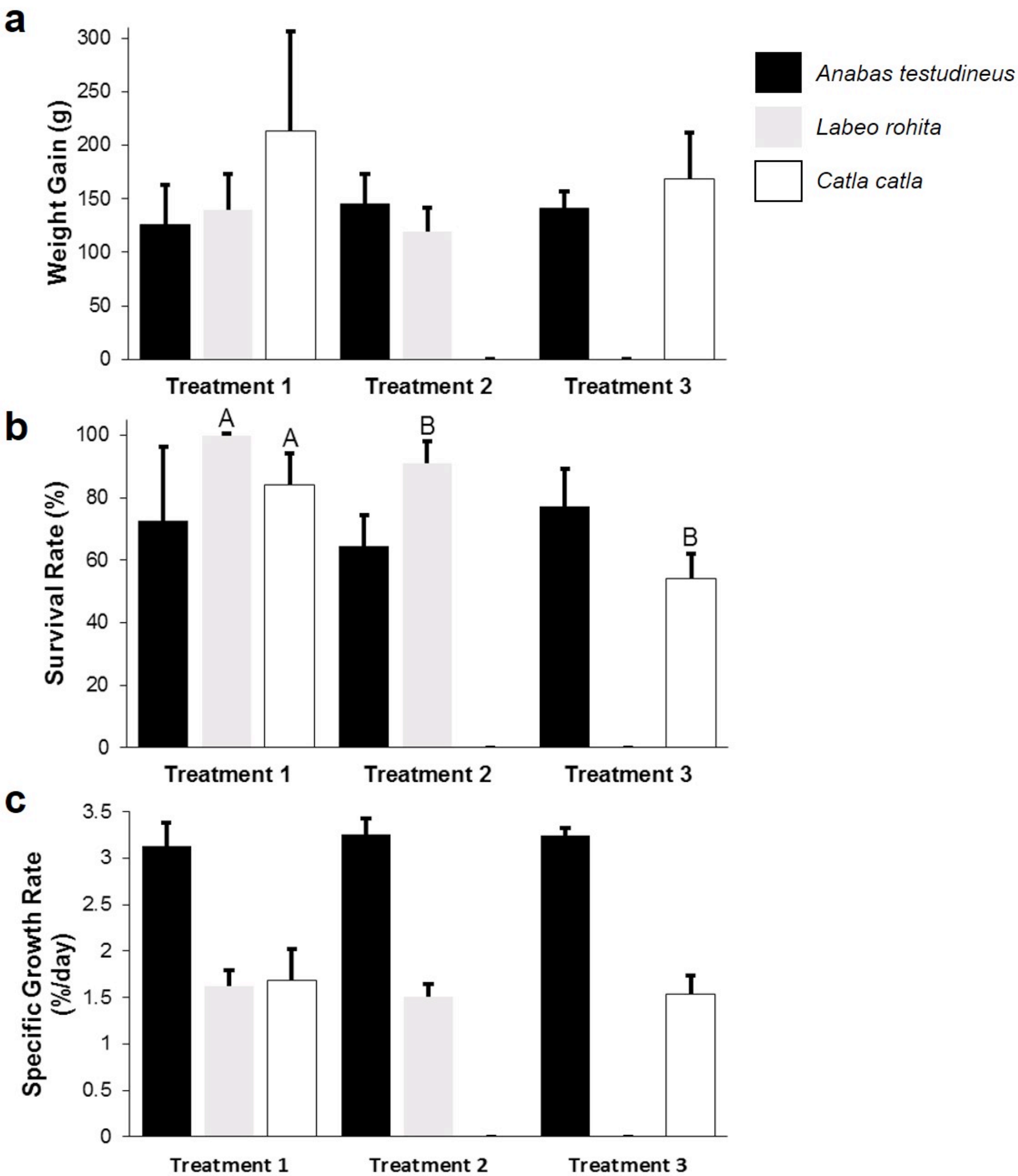

Figure 6. Weight gain, survival rate, and specific growth rate (SGR) for koi (*A. testudineus*), rohu (*L. rohita*), and catla (*C. catla*) in Study 2. There was no difference in weight gain (a) or SGR (c) for any species between treatments or for survival rate for koi (b). The survival rate for rohu in treatment 1 was significantly higher than treatment 2 and for catla in treatment 1 than in treatment 3. Values are mean ± SD. Different letters signify differences between treatments for the each respective species ($P < 0.05$).

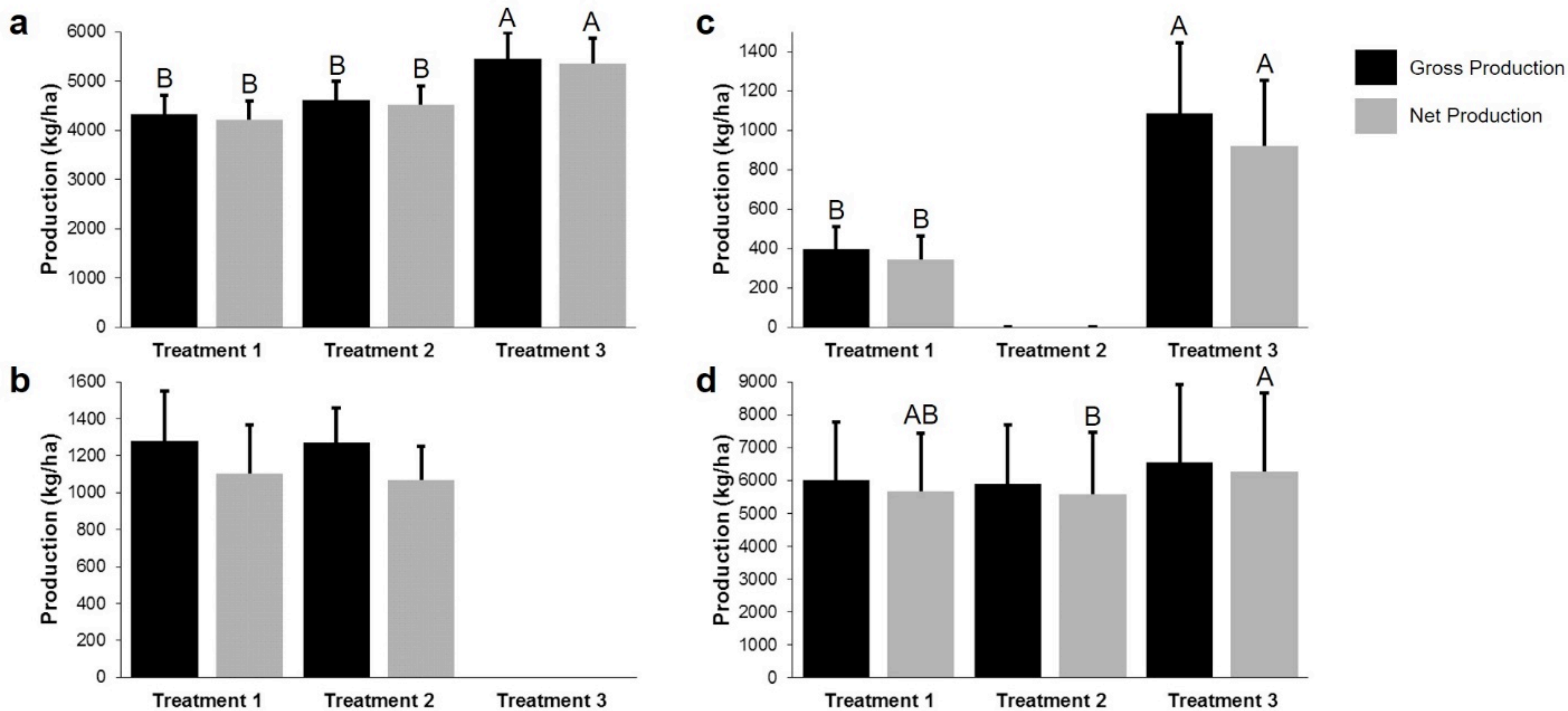

Figure 7. Gross and net production parameters for koi (*A. testudineus*, a), rohu (*L. rohita*, b), catla (*C. Catla*, c), and for combined species (d) in Study 2. Both parameters were significantly higher for koi in treatments where the koi were grown with catla only than when grown with rohu only or both catla and rohu. Catla grown at a stocking rate of 1.0 fish/m^2 resulted in production parameters that were significantly higher than when stocked at 0.2 fish/m^2. There was no significant difference in production of rohu between treatments. When combined, only net production was significantly different between treatment. Values are mean ± SD. Values with different letters are significantly different ($p < 0.05$).

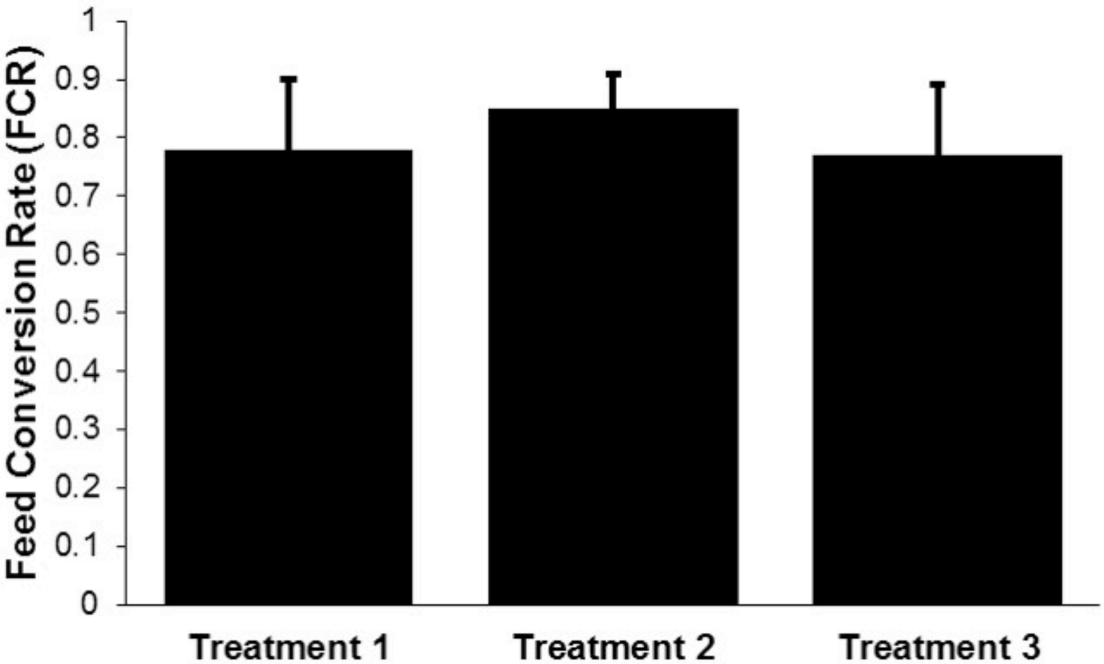

Figure 8. Combined feed conversion ratio (FCR) for koi (*A. testudineus*), rohu (*L. rohita*), catla (*C. catla*) in Study 2. There was no significant difference in overall FCR between treatments. Values are mean ± SD.