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Target-specific feeding strategies in carp-tilapia polyculture ponds: Effects of feed type and carp size

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ABSTRACT

In polyculture ponds, feeding strategies can affect the species-specific intake and utilisation of both supplementary feed and natural food, thereby influencing overall pond performance. This experiment tested the effects of two feeding strategies and two carp sizes at stocking in a two by two factorial design on fish performance and the food web in carp-tilapia polyculture ponds. The two strategies were feeding a low protein-floating feed in combination with a high protein-sinking feed (F_LS_H) and a high protein-floating feed in combination with a low protein-sinking feed (F_HS_L). The two carp sizes stocked were small (~22 g) and big (~135 g). Ponds with small carp were stocked with 144 carps, while ponds with big carp were stocked with 72 carps. Each pond was stocked with 16 similar sized tilapia. To give an equal nutrient input across all the ponds, the feed ration was determined for the small carp-ponds at the level of 18 $g.kg^{-0.8}.d^{-1}$, assuming 100 % survival. An equal amount of feed was applied to the big carp-ponds. The experiment was conducted in Bangladesh under very hot conditions associated with climate change: the average water temperature ranged between 33 and 35 ℃ in the afternoon (12:00–16:00 h). Results showed that the feeding strategy did not affect fish production and natural food availability ($P > 0.05$). However, survival was affected by feeding strategy ($P < 0.05$), carp size ($P < 0.001$) and their interaction ($P < 0.05$). Small carps, especially catla, suffered from higher mortality, but feeding a high protein-floating diet improved their survival. Despite lower survival and a higher body maintenance cost, ponds with small carps yielded a higher production than ponds with big carps. The underlying reason is possibly the stimulation of natural food production in the pond by the higher number of small fish present.

1. Introduction

The concept of pond polyculture involves growing different fish species together with partially overlapping or different feeding niches, with the aim that fishes will efficiently utilize the available natural food present in different compartments of the pond (Nekrasova et al., 2024; Milstein, 1992). Different feeds could be applied in such a way that they become available at various pond depths allowing efficient intake and utilisation by different species in the polyculture, but this is not practiced yet. Most likely because traditionally polyculture was practiced extensively with no or very little supplementary feeding. But today, modern polyculture is also practiced semi-intensively and with many farmers feeding compound feed (Chary et al., 2024). Akter et al. (2025) demonstrated that feeding on weight basis a 50:50 mixture of floating and sinking pellets with the same nutrient composition resulted in similar fish production when feeding 100 % floating pellets and higher fish production when feeding 100 % sinking pellets. Feeding the 50:50 mixture of floating and sinking feed also improved survival by lowering the interspecies competition. Apart from the 50:50 ratio, no other ratio of floating and sinking pellets was tested yet. To avoid applying an untested feed mixture ratio, this study applied the previous tested 50:50 ratio of floating and sinking pellets.

One important advantage of feeding a mixture of floating and sinking feed is that this allows to differentiate the nutrient input at various pond depths and to target specific species, especially when the feeds are formulated with a different nutrient composition. From a fish nutrition perspective, we expect that feeding a high protein-floating feed will enhance production of a surface feeder species, while a high protein-

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sinking feed will benefit a water column/bottom feeder species. However, in a pond, fish can compensate for lower nutrient intake from formulated feed by consuming natural food (Roy et al., 2023; Roy et al., 2022). Thus lowering the protein content in the feed does not necessarily reduces fish production. This makes it important to consider the effect of formulated feed not only on fish performance, but also on consumption and availability of natural food in ponds.

This study explored how manipulating the nutrient composition of floating and sinking feeds, while keeping the total nutrient input the same in all treatments, might influence the overall pond performance. Specific attention was given to total and species-specific fish production, natural food presence and water quality in each pond. To test this, two feeding strategies were applied- 1. Feeding a low protein-floating feed combined with high protein-sinking feed, and 2. Feeding a high protein-floating feed combined with low protein-sinking feed. The hypothesis was that the second feeding strategy will be best because the fish will consume more protein of a high protein-floating feed (Hossain et al., 2018; Yaqoob et al., 2010), leading to higher production, while the low protein-sinking feed will lead to less nitrogen accumulation on the bottom, keeping a healthier production environment (Avnimelech, 1999; Magondu et al., 2015).

For the practical application, it is also necessary to know if the applied feeding strategies return similar results when the size of fish is different. Fish size plays an important role in inter-species competition leading to an impact on survival, feed intake and growth of each species present in the system (Bautista-Vega et al., 2008). This study focuses on a four species combination of carp-tilapia polyculture comprising rohu (*Labeo rohita*), catla (*Catla catla*), silver carp (*Hypophthalmichthys molitrix*) and all-male Nile tilapia (*Oreochromis niloticus*). In carp-tilapia polyculture, at the beginning of the production cycle, carps and tilapia may have the same size but at a later stage the major carps will be bigger than tilapia (Akter et al., 2024). Therefore, besides the feeding strategy, this study also tested the effect of carp size on fish production and natural food availability in carp-tilapia polyculture ponds.

2. Materials and methods

2.1. Experimental design

The main aim of this experiment was to understand if, a fish species group or species in a carp-tilapia polyculture pond can be targeted fed, resulting in enhanced production by providing a different nutrient input at different pond depths. To supply nutrients at different pond depths, floating and sinking feed were mixed at a 1:1 ratio, thereby ensuring that the total nutrient content fed to each pond was the same. To differentiate the amount of nutrients available at different pond depths, two feeding strategies were defined. The first strategy involved delivering less protein and more carbohydrates at the pond surface, while the opposite was done at the pond bottom. The second strategy involved delivering more protein and less carbohydrates at the pond surface, with the reverse applied at the pond bottom. To implement these strategies, two diets were formulated: one with low protein and high carbohydrate content and the other with high protein and low carbohydrate content (Tables 1 and 2).

Both diets were produced in two different forms: floating and sinking. Shafique feed mills Ltd. in Bangladesh produced these four experimental diets: F_L, floating feed with a low protein content; F_H, floating feed with a high protein content; S_L, sinking feed with a low protein content; and S_H, sinking feed with a high protein content. With these four pellet types two feeding strategies were applied: strategy F_LS_H, in which ponds were fed F_L and S_H pellets; and strategy F_HS_L, in which ponds were fed F_H and S_L pellets. At both feeding strategy the ratio between both types of pellets (on weight basis) given was equal (1:1), thereby supplying equal amounts of nutrients to each pond.

With the F_LS_H strategy a larger share of the daily protein supply reached at the bottom section of the pond, whereas with the F_HS_L strategy more protein remained at the surface of the pond. Regarding the daily carbohydrate input, the majority was available at the surface and bottom of the pond with the F_LS_H and F_HS_L feeding strategy, respectively.

Table 1
Ingredient composition of the two formulas used for having diets with low and high protein content.

	Low protein	High protein
Protein sources (%)		
Soyabean meal	6.60	17.30
Rape seed meal	6.60	17.30
Fishmeal	8.70	23.10
L-Lysine HCl	0.60	0.60
DL-Methionine	0.30	0.30
L-Threonine	0.10	0.10
Carbohydrate sources (%)		
Maize	20.70	10.40
Wheat flour	20.70	10.40
Rice bran de-oiled	30.30	15.10
Basal part (%)		
Fishoil	3.00	3.00
Chalk ($CaCO_3$)	0.25	0.25
Monocalcium phosphate	1.50	1.50
Premix DSM 2011	0.25	0.25
Pegabind	0.40	0.40
Total	100	100

Both formulated diets were produced as floating (F) and sinking (S) pellets.

Table 2
Analysed chemical composition of the experimental carp-tilapia diets differing in crude protein content, which were produced as both as floating (F) and sinking (S) pellets.

		Diets			
		Floating		Sinking	
Parameters	Unit	F_L	F_H	S_L	S_H
Dry matter (DM)	$g.kg^{-1}$	921	925	885	887
Crude protein	$g.kg^{-1}$ DM	229	285	229	350
Crude fat	$g.kg^{-1}$ DM	64	55	43	48
Ash	$g.kg^{-1}$ DM	96	109	98	112
Carbohydrate	$g.kg^{-1}$ DM	531	476	514	377
Crude fiber	$g.kg^{-1}$ DM	48	41	64	65

F= floating feed, S= sinking feed, subscripts indicate crude protein content of the pellets - L= low and H= high.

The experiment lasted 56 days from 16 July to 09 September 2022, and had a two by two factorial design: two feeding strategies (F_LS_H versus F_HS_L); and two size classes of carps at stocking (small, average weight 22 g versus big, average weight 135 g), resulting in four treatments. With the limitation of the experimental pond size (45 m^2), it was not possible to test carps larger than ~150 g, because stocking larger fish (around twenty individuals per carp species, in total sixty or more carps) is likely to cause exceeding the pond's carrying capacity during the experiment. In addition, obtaining carp species with the same average weight to stock the ponds was difficult. Therefore, the average weight of each carp species stocked was slightly different. The resulting average weight of all carps stocked was 135 g. In the experiment, twenty four ponds, distributed in two adjacent rows with 12 ponds per row, were used. From a previous experiment, it was known that the experimental pond area is homogenous. Therefore, the treatments were randomly assigned, resulting in six replicate ponds per treatment.

The floating pellets (F_L and F_H) were produced by extrusion and the sinking pellets (S_L and S_H) by steam pelleting, using a 2 mm die resulting in 3 mm pellets. The floatability of the floating pellet types (F_L and F_H) over time was tested in glass jars filled with water. After 1 h still 100 % of F_L and F_H pellets were floating, after 4 h 100 % and 99 %, after 6 h 100 % and 88 % and after 8 h, 100 % and 67 % of the F_L and F_H pellets, respectively. Fish were provided by a supplier who collected them from local farms and hatcheries. The number and weight of fish per pond at stocking are presented in Table 3. The mean weights at stocking of small catla, rohu and silver carp were 19, 21 and 28 g, respectively and of big carps 143, 91 and 171 g, respectively.

2.2. Pond preparation

This study was done in a dedicated experimental pond facility at Khulna University, Bangladesh. Twenty-four ponds, each measuring 45 m^2, and around one meter deep were used for this experiment. Pond preparation began two weeks before the experiment. First, all aquatic vegetation inside and outside the ponds was removed. Next, the ponds were disinfected by applying bleaching powder at a rate of 30 $g.m^{-2}$ of the pond surface (Boyd, 2019; Kumar, 1992). Due to the low-lying location of the pond facility, sun-drying of the pond bottom was not practical. After applying the bleaching powder, a ten-day waiting period was implemented to eliminate toxicity. Subsequently, each pond received fertilisation with 1 g of urea and 2 g of triple super phosphate per square meter pond surface (Rakocy and McGinty, 1990).

Five days later, fish were stocked in each of the twenty-four ponds. Each pond was stocked with a four species polyculture combination, comprising catla, rohu, silver carp and Nile tilapia. Catla and silver carp are both surface feeders and thus occupy the surface to mid water layers of the pond. Rohu prefers the middle to bottom water layers, and tilapia explores all pond layers. The number of fish stocked in small and big carp-ponds was different, as detailed in Table 3.

Each pair of ponds was aerated with one Resun LP-100 air pump, dividing the outflow equally. Aeration was homogenously distributed around the ponds using sixteen air stones. Throughout the experiment, all ponds received an equal amount of aeration with the aim to prevent the dissolved oxygen (DO) level from dropping below 4 $mg.L^{-1}$.

2.3. Feeding and feed intake monitoring

All 24 ponds received an equal amount of feed with the same nutrient content. However, half of the ponds (12) were fed according to the F_HS_L strategy, while for the other half, the F_LS_H strategy was followed. The daily feed ration was pre-determined based on the metabolic body weight of the fish. The feeding level was set at 18 $g.kg^{-0.8}.d^{-1}$, as we knew from beforehand based on our previous study (Akter et al., 2025) that the pond system will do well with this chosen feeding level, and the amount of aeration provided. The daily amount of feed given increased based on a predicted growth assuming a FCR of 1.2. Silver carp were excluded from the feed ration calculation due to their known preference in ponds for natural food (Cremer and Smitherman, 1980; Dong and Li, 1994). The number of fish used to calculate the daily feed ration, was 112 (48 fish of each of the two major carp species and 16 tilapia), with an average stocking weight of 20 g. The feed ration calculated for the small carp-ponds was used in all ponds, regardless of carp size, to ensure equal feed input to all ponds. By calculating the feed ration for small carp-ponds, we indirectly made sure that there would be sufficient feed in the large carp-ponds during the experiment, as large fish would have a lower feed demand based on their metabolic weight, than small fish. In total, each pond was fed 10 kg feed (5 kg floating and 5 kg sinking pellets).

Table 3
The number and weight of fish at stocking.

	Unit	Small carp-ponds	Big carp-ponds
Carp weight	g	22	135
Tilapia weight	g	20	20
Number of carps per pond		144	72
Number of tilapia per pond		16	16
Total number of fish per pond		160	88
Stocked biomass per pond	kg	3.6	10

Forty percent of the daily feed ration was provided in the morning, while the remaining sixty percent was fed in the afternoon. Intake of floating feed was monitored quantitatively by recording the moment of feed delivery and the moment when no more pellets were seen at in the pond surface. To determine the moment when all floating pellets were consumed, ponds were visually inspected every 15 min by walking along the pond dykes. The feed intake time was calculated by subtracting the moment of feed delivery from the moment when no pellets were seen at the pond surface. The feed intake rate per pond (in $g.min^{-1}$) was calculated by dividing the amount of delivered feed by the feed intake time.

2.4. Water quality

Water quality parameters in each pond were monitored three times a day at 08:00, 12:00 and 16:00 h. Water temperature, salinity, pH and electric conductivity (EC) were monitored from the first day of the experiment using a multiparameter YSI 5200 A meter (Neil et al., 2013). The DO was monitored using a Lutron PDO-519 dissolved oxygen meter from day 17 of the experiment onwards, as during the preceding period, the DO meter was not working. The total dissolved solid (TDS) concentration was calculated from EC (Taylor et al.,2018). Concentrations of TAN (NH_4-N) were measured at days 28, 42 and 56 using a freshwater Hanna test kits for ammonia (HI 3824).

2.5. Phytoplankton, zooplankton and benthic macroinvertebrates monitoring

Phytoplankton and zooplankton samples were collected at two-week intervals, beginning from experimental day 14. Zooplankton and phytoplankton were collected by filtering 45 L water from each pond (15 L water from each of three equally distant locations in the pond) through forty-five and fifteen micro-meter plankton nets, respectively (Suthers et al., 2019). Then the concentrated phytoplankton and zooplankton samples were preserved in 5 % buffered formalin (Mukherjee et al., 2014; ASTM International, 2019). Later, each phytoplankton and zooplankton sample was analysed by examining a 1 ml representative subsample in a Sedgwick-Rafter (S-R) chamber under a microscope. Of the 1000 1-mm^3 cells in the S-R chamber, 100 and 200 randomly selected cells were examined, for phytoplankton and zooplankton, respectively to count and identify up to genus level (Suthers et al., 2019). In case of colonial algae every cell was counted except for the genera *Aphanocapsa*, *Scenedesmus*, *Pediastrum*, and *Gomphosphaeria*, where the whole colony was counted as one. In case of filamentous algae, each filament was counted as one. The number of phytoplankton cells counted in 100 S-R cells was multiplied by 10 to calculate the number of cells present in 1 ml. Similarly, the number of zooplankton counted in 200 cells of S-R chamber was multiplied by 5 to calculate number present in 1 ml. Damaged organisms were counted when only a tiny fraction was missing, to avoid double counting. The abundance ($ind.L^{-1}$) of phytoplankton and zooplankton was calculated as $A = \frac{N \times C}{V}$, where, abundance A = number of plankton cells in 1 L pond water; N = number of plankton cells found in 1 ml sample; C = volume of concentrated plankton sample (ml); and V = volume of the filtered pond water sample (L). The number of plankton genera present in each plankton sample was reported as the diversity ($genera.L^{-1}$).

For benthic macroinvertebrates collection, mud samples were collected from the pond bottom at two-week intervals, starting from experimental day 14, from four replicate ponds of each treatment. Mud samples were collected by LaMotte bottom sampling dredge (290 cm^2 surface collection area) by blocking its ventilation holes with duct tape to prevent sample loss (Branstrator et al., 2006). In each pond, bottom mud samples were collected from three equal distant locations and combined to create a composite sample. Then the composite mud sample was sieved through 0.5 mm mesh with water (Standard operating procedure, 2003). Retained benthic macroinvertebrates in the sieve were collected and preserved in a plastic jar containing 10 % buffered formalin. The collected organisms were identified under a microscope up to the family level and counted to calculate their abundance (ind. m^{-2}) and diversity (family.m^{-2}). The abundance of benthic macroinvertebrates was estimated from the equation: $A = \frac{Y \times 10000}{3a}$, where abundance, A = number of benthic organisms available per square meter of pond bottom sediment; Y = counted number of benthic organisms in each composite sample from each pond, a = area of the sampling dredge. Multiplication by 10,000 was done to convert the dredge area from cm^2 to m^2. The number of families identified in each sample was reported as diversity (family.m^{-2}).

Unfortunately, due to logistics issues, we could not process six out of the sixteen samples collected on experimental day 56.

2.6. Proximate composition analysis of fish and feed

Feed samples were taken at the beginning of the experiment and stored at −20 °C. For whole body composition analysis, at stocking 15 fish per species were randomly selected from the base populations. At harvesting, five fish per species per pond were randomly selected. Fishes were euthanized at sampling and stored at −20 °C for further analysis. Later, feed and fish samples were sent to an external lab (Department of Marine Bioresource Science, Chattogram Veterinary and Animal Sciences University, Bangladesh) and analysed for dry matter, crude protein, crude fat and ash content according to the AOAC, (1990). Additionally, feed samples, were analysed for crude fiber content by the ceramic fiber filter method (AOAC, 1990).

2.7. Fish performance calculation

Fish were harvested by netting, followed by draining the ponds. In each pond, all harvested fish were counted and bulk weighed per fish species. The survival percentage per fish species in each pond was calculated by dividing the number of harvested fish by the number of stocked fish and multiplied by hundred. Similarly, the overall survival percentage per pond was determined using the total number of fish stocked and harvested. The biomass gain per species was calculated for each pond by subtracting the stocked biomass from the harvested biomass and similarly the total fish biomass gain by subtracting the sum of the stocked biomass of all species from the sum of the harvested biomass of all species. The feed conversion ratio (FCR) was calculated by dividing the amount of delivered feed by the total fish biomass gain in each pond. The absolute growth rate per fish for each species (g.d^{-1}) was determined by dividing the individual weight gain (in grams) by the duration of the experiment (days).

2.8. Statistical analysis

Data were analysed using R studio version 4.3.3. Parameters measured at the pond level (harvested biomass, total biomass gain, survival, FCR, feed intake time and rate) were analysed by two-way ANOVA for the effects of feeding strategy (FS), carp size (CS) and their interaction (FS×CS). Harvested biomass, total biomass gain and FCR data were transformed, respectively, by rank, sqrt(x + 0.5) and ln (x) to achieved normality of residuals. One data point of FCR was omitted in the statistical analysis as it was a negative value. Parameters measured at species level (survival, biomass gain, AGR and body composition parameters) were analysed by mixed ANOVA with the packages 'lme4' (Douglas et al., 2015), 'afex' (Singmann et al., 2024) and 'ez' (Lawrence, 2016). The mixed ANOVA tested for the effect of FS, CS (between pond factors), species (Sp; within pond factor) and their interactions (FS×CS, FS×Sp, CS×Sp and FS×CS×Sp). Regarding water quality data, the 56-day experimental period was divided into four 2-week quarters. For each water quality parameter, the data were averaged per quarter and analysed by mixed ANOVA. This analysis considered FS and CS as between-pond factors and experimental quarter (EQ) as a within-pond factor and examined their effects and interactions. The abundance and diversity of phytoplankton and zooplankton were analysed by mixed ANOVA and of benthic macroinvertebrates were analysed by mixed model to test the effect of FS, CS, time (T) and their interactions. The 6 missing benthos abundance and benthos diversity samples were not included in the mixed model analysis. In the cases of significant effects, means were compared by Tukey's test with the packages 'emmeans' (Lenth, 2024) and 'multcomp' (Hothorn et al., 2008). Graphs were prepared in R using the packages 'tidyverse' (Wickham et al., 2019), 'ggplot2' (Wickham, 2016), 'ggtext' (Wilke and Wiernik, 2022) and 'scales' (Wickham et al., 2023). For all statistical test, $P < 0.05$ was considered significant different.

3. Results

3.1. Fish performance

Overall fish production was unaffected by feeding strategy ($P > 0.05$, Table 4), as indicated by harvested biomass, total biomass gain, and feed conversion ratio (FCR). However, carp size at stocking influenced all these performance parameters ($P < 0.001$). Ponds stocked with small carps had higher biomass gain (3.0 kg) and lower FCR (4.0) than ponds stocked with big carps (1.1 kg and 13.1, respectively; Table 4). Fish survival at pond level was affected by carp size ($P < 0.001$), feeding strategy ($P < 0.05$), and their interaction ($P < 0.05$; Table 4). Ponds stocked with big carps had a high survival (94 %) and had a similar survival at both feeding strategies (Fig. 1, Supplementary Table S1). In contrast, ponds stocked with small carps had lower survival (80 %) and the feeding strategy resulted in different survival. In the small carp-ponds, feeding floating pellets with a high protein content and sinking pellets with a low protein content (the F_HS_L strategy), showed improved survival (86 %) compared to feeding according to the F_LS_H strategy (75 %, Fig. 1, Supplementary Table S1).

In the studied carp-tilapia polyculture, survival ($P < 0.001$), biomass gain ($P < 0.001$) and absolute growth rate (AGR, $P < 0.001$) differed between species (Table 5, Supplementary Table S2). Survival was highest in rohu (98 %) and tilapia (95 %) followed by silver carp (87 %) and was lowest in catla (73 %; Table 5). Regarding survival, there was an interaction effect present between species and carp size at stocking ($P < 0.001$). Survival was similar at both carp size treatments for tilapia, silver carp and rohu, but survival of catla differed between the carp size treatments (Fig. 2 A). Survival of small catla was lower than survival of big catla (51 versus 95 %). Furthermore, the variability in survival of catla between ponds was largest for treatments stocked with small carps. There was also an interaction effect present between feeding strategy and species on survival ($P < 0.05$; Table 5). Similar as for the carp size species interaction effect, only catla was affected by feeding strategy. Feeding more protein to the surface layer of the pond under feeding strategy F_HS_L resulted in a higher survival compared to feeding more protein in the bottom layer of the pond under feeding strategy F_LS_H (81 versus 65 %; Fig. 2B).

Biomass gain differed between fish species ($P < 0.001$). Biomass gain was positive and highest in rohu and tilapia. For catla and silver carp biomass gain was negative, −0.16 and −0.03 kg, respectively (Table 5). The biomass loss in catla and silver carp resulted from their low survival. The absolute growth rate (AGR) was different between species ($P < 0.001$), being highest in tilapia (1.21 g.d^{-1}) followed by rohu (0.68 g.d^{-1}), catla (0.15 g.d^{-1}) and silver carp (0.12 g.d^{-1}) (Table 5). The feeding strategy did not influence species-specific biomass gain and AGR, i.e., no interaction effect between feeding strategy and species ($P > 0.05$, Table 4). Whereas the interaction between fish species and carp size treatment was present for both biomass gain and AGR ($P < 0.01$; Table 5). The carp size had no impact on biomass gain and AGR of catla (Figs. 3A and 3B). Biomass gain was higher for rohu, silver

Table 4
Main effects of feeding strategy (FS) and carp size (CS) on fish performance at pond level.

Parameters	Unit	FS		CS		SEM	Significant effects
		F_LS_H	F_HS_L	Small	Big		
No. of fish stocked		124	124	160	88	—	NA
Stocked biomass	kg	6.79	6.80	3.55^b	10.04^a	0.009	CS***
Harvested biomass	kg	8.78	8.97	6.58^b	11.17^a	0.406	CS***
Total biomass gain	kg	1.99	2.16	3.02^a	1.13^b	0.402	CS***
Feed delivered	kg	10.0	10.0	10.0	10.0	—	—
Survival per pond	%	85^b	90^a	80^b	94^a	1.54	CS***, FS*,FS×CS*
FCR	$g.g^{-1}$	10.8	6.3	4.0^b	13.1^a	5.97	CS**

F_LS_H = Low protein-floating feed and high protein-sinking feed; F_HS_L = High protein-floating feed and low protein-sinking feed; SEM= Standard error of mean. All numerical values presented in the table are from original data, while statistical results for harvested biomass, total biomass gain and FCR came from transformed data, as those were transformed to meet the normality before statistical analysis. Two way ANOVA was done to test the main effect of FS and CS and their interaction effect. Only significant effects are shown in the last column. When a significant effect was found, mean comparisons were done using Tukey's test. Factor values for each parameter, without a letter in common are different ($P < 0.05$). P values: * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$; Main or interaction effects which are not shown in the column 'Significant effects', were not significant ($P > 0.05$).

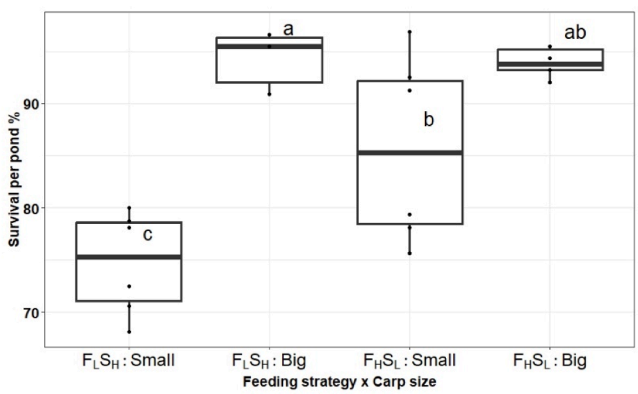

Fig. 1. Survival averaged over all four species as affected at the four experimental treatments, showing the interaction effect between feeding strategy and carp size at stocking. F_LS_H = Low protein-floating feed and high protein-sinking feed; F_HS_L = High protein-floating feed and low protein-sinking feed; small = ponds stocked with small carps, Big = ponds stocked with big carps; Mean comparison was done using Tukey's test ($P < 0.05$). Survival in treatments without a letter in common are different from each other ($P < 0.05$).

carp and tilapia in ponds stocked with small carps than in ponds stocked with big carps (Fig. 3A). Similarly, a higher AGR was observed for silver carp and tilapia in ponds stocked with small carps than ponds with big carps while a similar growth rate was observed for rohu and catla with both carp size treatments (Fig. 3B).

3.2. Feed intake monitoring

Feed intake time and rate were influenced by carp size ($P < 0.05$ and $P < 0.01$, respectively) but not by feeding strategy ($P > 0.05$, Table 6). Ponds stocked with big carps had a shorter feed intake time (0.9 h) and a higher feed intake rate (1.0 $g.min^{-1}$) than ponds stocked with small carps (1.1 h and 0.8 $g.min^{-1}$, respectively) (Table 6).

3.3. Fish body composition

Carp size influenced dry matter ($P < 0.01$), crude protein ($P < 0.01$) and crude fat ($P < 0.001$) content of fish at harvest, while ash content was not affected ($P > 0.05$, Table 7). Compared to fish in small carp-ponds, fish in big carp-ponds had a higher dry matter (315 versus 305 $g.kg^{-1}$) and crude fat content (68 versus 53 $g.kg^{-1}$). In contrast, the crude protein content in fish from big carp-ponds was lower than that in the small carp-ponds (170 versus 176 $g.kg^{-1}$). However, the differences in dry matter and protein content were small between carp size treatments.

Dry matter content varied among the species ($P < 0.001$), being highest in tilapia (337 $g.kg^{-1}$) and comparatively lower but similar in rohu (307 $g.kg^{-1}$), catla (302 $g.kg^{-1}$) and silver carp (295 $g.kg^{-1}$, Table 7, Supplementary Table S3). Also crude fat and ash content varied among species ($P < 0.001$ and $P < 0.01$, respectively). The effect of carp size on each species showed that fat content of big carps was higher than of small carps (Fig. 4A). In contrast, big catla and rohu had a lower ash content than small catla and rohu (Fig. 5A). Silver carp had equal ash content at both sizes, whereas tilapia exhibited higher ash content in ponds with big carps compared to ponds with small carps (Fig. 5A).

Feeding strategy affected the overall crude fat ($P < 0.01$) and ash content ($P < 0.01$) in fish while dry matter and crude protein content were not affected ($P > 0.05$, Table 7). The crude fat content in fish fed with high-protein floating and low-protein sinking feed (F_HS_L - 63 $g.kg^{-1}$) was higher than that in fish fed with low-protein floating and high-protein sinking feed (F_LS_H - 58 $g.kg^{-1}$).

The effect of feeding strategy on fat content of each species showed that the above-mentioned effect was true for tilapia while carps

Table 5
Main effects of species (Sp) on fish performance.

Parameters	**Unit**	**Species (Sp)**				**SEM**	**Significant effects**
		Catla	**Rohu**	**Silver carp**	**Tilapia**		
Survival	%	73^c	98^a	87^b	95^a	2	Sp***, CS×Sp***, FS×Sp*
Biomass gain	kg	-0.16^b	1.26^a	-0.03^b	1.01^a	0.1	Sp***, CS×Sp***
AGR	$g.d^{-1}$	0.15^c	0.68^b	0.12^c	1.21^a	0.06	Sp***, CS×Sp**

F_LS_H = Low protein-floating feed and high protein-sinking feed; F_HS_L = High protein-floating feed and low protein-sinking feed; AGR = Absolute growth rate; SEM= Standard error of mean; FS = feeding strategy; CS = carp size (CS). Mixed ANOVA was done to test main effect of FS, CS, FS×CS, Sp, FS×Sp, CS×Sp, and CS×FS×Sp. Main effects of FS and CS are shown in Table 4 and thus not shown here. Only significant effects of Sp and interaction effects of Sp with FS and CS are shown in the last column. When a significant effect was found, mean comparisons were done using Tukey's test. Per row, Sp values for each parameter, without a letter in common are different ($P < 0.05$). P values: * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$; Main or interaction effects that are not shown in the last column, were not significant ($P > 0.05$).

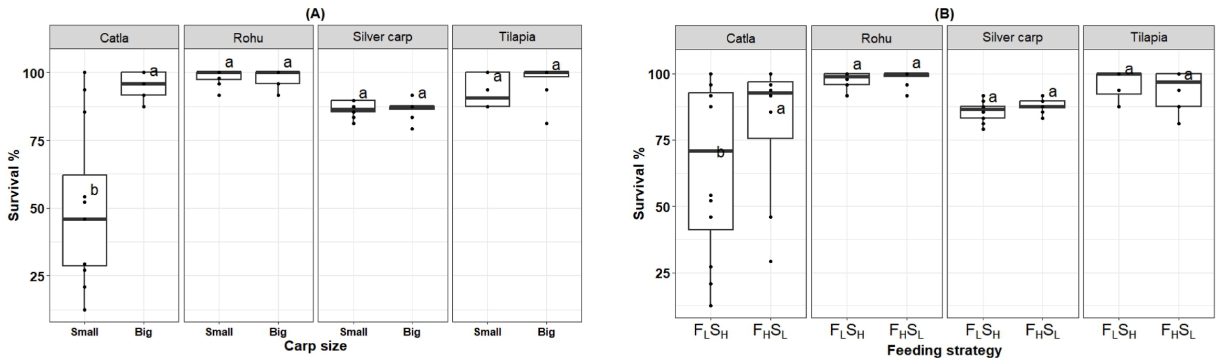

Fig. 2. (A) effect of carp size on survival of each species in ponds, (B) effect of feeding strategy on survival of each species in ponds, F_LS_H = Low protein-floating feed and high protein-sinking feed; F_HS_L = High protein-floating feed and low protein-sinking feed. Mean comparisons were done using Tukey's test (P < 0.05). For each species, treatments without a letter in common are different from each other (P < 0.05).

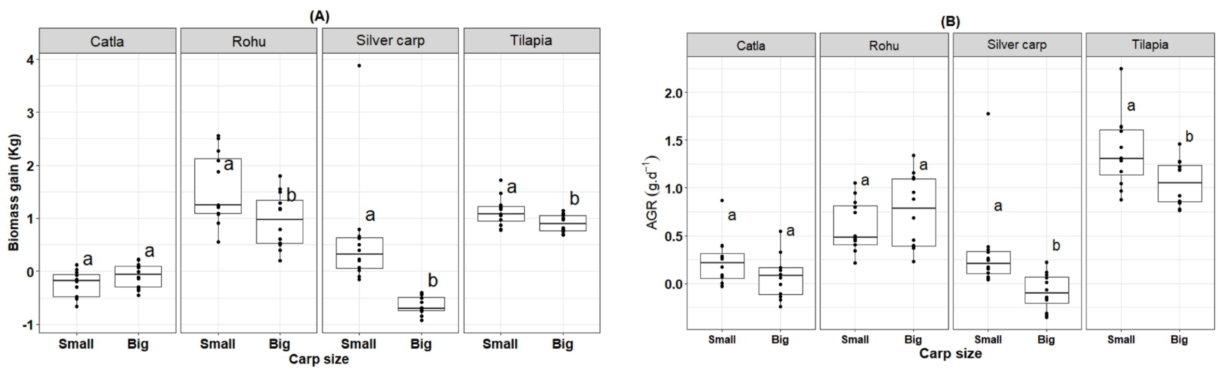

Fig. 3. Effect of carp size on biomass gain (A) and absolute growth rate (AGR) (B) of each species in the polyculture ponds, Mean comparisons were done using Tukey's test (P < 0.05). For each species, treatments without a letter in common are different from each other (P < 0.05).

remained unaffected (Fig. 4B). The ash content showed the opposite of the fat content being higher in fish fed under the F_LS_H strategy (66 g. kg^{-1}) than in fish fed under the F_HS_L strategy (63 g.kg^{-1}, Table 7). The effect of feeding strategy on ash content of each species showed that the ash content of rohu and catla was higher when fed under the F_HS_L strategy than under the F_LS_H strategy (Fig. 5B). Silver carp had equal ash content under both feeding strategies while tilapia had higher ash content when fed under the F_LS_H strategy than under the F_HS_L strategy

Table 6
Main effects of feeding strategy (FS) and carp size (CS) on feed intake time and rate of the floating pellets per pond.

		FS		CS			Significant effects
Parameters	Unit	F_LS_H	F_HS_L	Small	Big	SEM	
Feed intake time	hour.pond^{-1}	1.0	1.0	1.1^a	0.9^b	0.08	CS*
Feed intake rate	g.min^{-1}.pond^{-1}	0.9	0.9	0.8^b	1.0^a	0.05	CS**

F_LS_H = Low protein-floating feed and high protein-sinking feed; F_HS_L = High protein-floating feed and low protein-sinking feed; SEM= Standard error of mean. Two way ANOVA was done to test the main effect of FS and CS and their interaction effect. Only significant effects are shown in the last column. When a significant effect was found, mean comparisons were done using Tukey's test. Factor values for each parameter, without a letter in common are different (P < 0.05). P values: * P < 0.05, ** P < 0.01; Main or interaction effects that are not shown in the last column, were not significant (P > 0.05).

Table 7
Main effects of feeding strategy (FS), carp size (CS) and fish species (Sp) on fish body composition on fresh basis (g.kg^{-1}).

	FS		CS			Sp					
Parameters	F_LS_H	F_HS_L	Small	Big	SEM	Catla	Rohu	Silver carp	Tilapia	SEM	Significant effects
Dry matter	310	310	305^b	315^a	2.14	302^b	307^b	295^b	337^a	3.8	CS**, Sp***
Crude protein	174	172	176^a	170^b	1.15	171	172	177	172	2.4	CS**
Crude Fat	58^b	63^a	53^b	68^a	1.1	54^c	62^b	38^d	88^a	1.5	CS***, FS**, Sp***,CSxSp***, FSxSp*
Ash	66^a	63^b	64	64	0.8	65^a	59^b	68^a	65^{ab}	1.4	FS**, Sp**,CSxSp***, FSxSp***

F_LS_H = Low protein-floating feed and high protein-sinking feed; F_HS_L = High protein-floating feed and low protein-sinking feed; SEM= Standard error of mean. Mixed ANOVA was done to test main effect of FS, CS, FS×CS, Sp, FS×Sp, CS×Sp and CS×FS×Sp. When a significant effect was found, mean comparisons were done using Tukey's test. Per row, factor values without a letter in common are different (P < 0.05). P values: * P < 0.05, ** P < 0.01, *** P < 0.001; Main or interaction effects that are not shown in the last column, were not significant (P > 0.05).

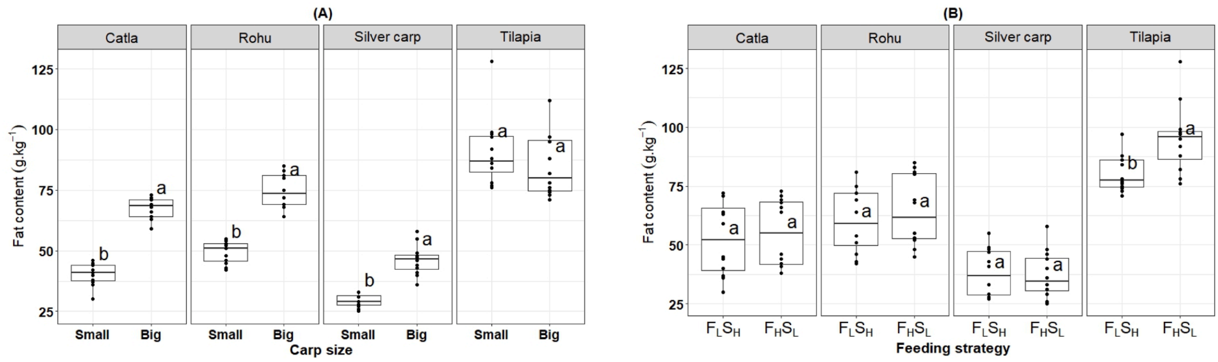

Fig. 4. Effect of carp size (A) and feeding strategy (B) on fat content of each species in experimental polyculture ponds. F_LS_H = Low protein-floating feed and high protein-sinking feed; F_HS_L = High protein-floating feed and low protein-sinking feed. Mean comparisons were done using Tukey's test. For each species, treatments without a letter in common are different from each other ($P < 0.05$).

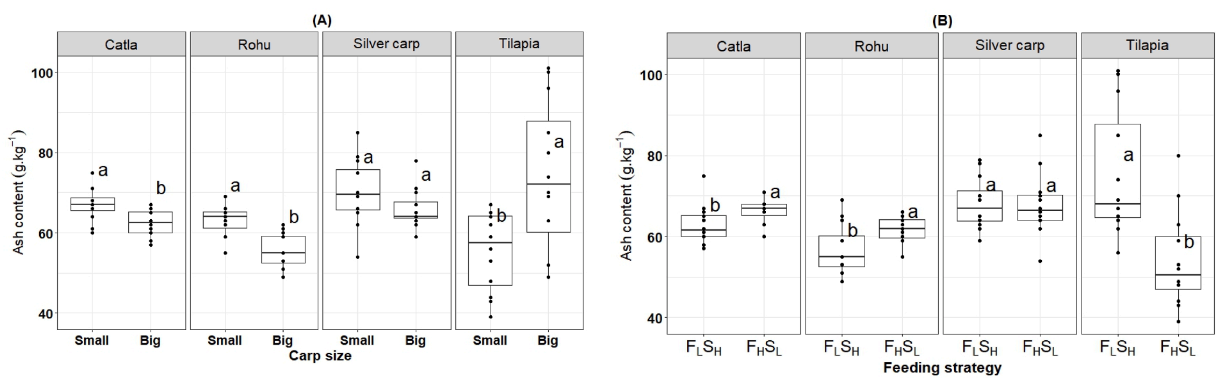

Fig. 5. Effect of carp size (A) and feeding strategy (B) on ash content of each species in experimental polyculture ponds. F_LS_H = Low protein-floating feed and high protein-sinking feed; F_HS_L = High protein-floating feed and low protein-sinking feed. Mean comparisons were done using Tukey's test. For each species, treatments without a letter in common are different from each other ($P < 0.05$).

(Fig. 5B).

3.4. Water quality monitoring

All water quality parameters were unaffected by carp size and feeding strategy ($P > 0.05$) but differed between the experimental quarters ($P < 0.05$; Table 8, Supplementary Table S4). Throughout the experiment, water quality in the experimental ponds remain within the acceptable range for carp-tilapia polyculture (Abdel-Tawwab et al., 2014; Banerjea, 1967; Deb et al., 2020; Keshavanath et al., 2012). However, the average temperature observed during the experiment was high (>33 °C, Table 8), especially during the second half of the day. Between 12:00 and 16:00 h the water temperature ranged between 33 and 35℃ (Fig. 6). With time, salinity decreased from 1.4 to 1.1 ppt during the experiment. The pH fluctuated between 8.3 and 8.4 during the experiment without a clear trend over time. The DO concentration fluctuated between 4 and 6 mg.L^{-1} which may be an effect of variation in aeration time. The total dissolved solids concentration decreased with time from 1839 to 1572 mg.L^{-1}. The total ammonia nitrogen (TAN) concentration fluctuated between 0.6 and 0.8 mg.L^{-1} throughout the experiment (Table 5.8).

3.5. Natural food web monitoring

The abundance and diversity of phytoplankton and zooplankton were not affected by main effects of carp size and feeding strategy

Table 8
Pond water quality over time (i.e., 2-wk experimental quarters, EQ).

Parameters	Unit	Experimental quarters (EQ)					Significant effects
		1	2	3	4	SEM	
Temperature	°C	33.5^{b}	33.2^{b}	32.0^{c}	34.1^{a}	0.09	EQ***
Salinity	ppt	1.4^{a}	1.3^{b}	1.1^{c}	1.1^{c}	0.06	EQ***
pH		8.37^{ab}	8.41^{a}	8.31^{b}	8.37^{ab}	0.02	EQ*
DO	mg.L^{-1}	—	3.9^{c}	4.9^{b}	5.7^{a}	0.03	EQ***
TDS	mg.L^{-1}	1839^{a}	1767^{b}	1600^{c}	1572^{d}	79	EQ***
Total ammonia nitrogen (TAN)	mg.L^{-1}	—	0.8	0.6	0.7	0.12	—

CS= Carp size, FS = Feeding strategy of floating-sinking mixed diet, EQ = Experimental quarter. Mixed ANOVA was performed to test the main effect of CS, FS, EQ and their interaction effects CS×FS, CS×EQ, FS×EQ and CS×FS×EQ. Only significant effects are shown in the last column. P values: *$P < 0.05$, *** $P < 0.001$. When a significant effect was found, mean comparisons were done using Tukey's test. EQ values without a letter in common are different ($P < 0.05$). Main and interaction effects that are not shown in the last column, were not significant ($P > 0.05$). Total ammonia nitrogen (TAN) was recorded on experimental day 28, 42 and 56.

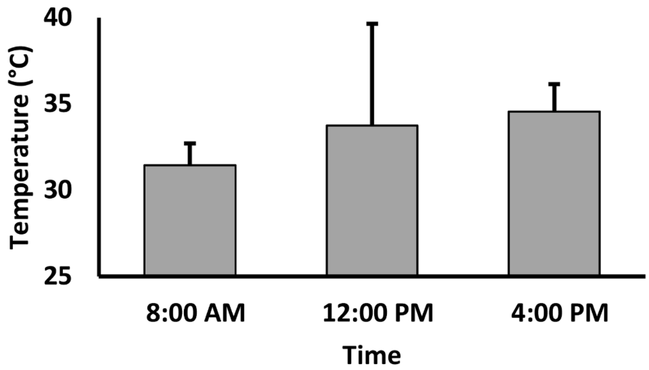

Fig. 6. Average temperature in three different time point of experimental days. Data were averaged over days and experimental treatments.

($P > 0.05$; Table 9, Supplementary Table S5). However, time affected the observed abundance and diversity of phytoplankton in the experimental ponds ($P < 0.05$; Table 9). Phytoplankton abundance increased steadily from 2769 ind.L^{-1} at day-14–7300 ind.L^{-1} at day-56 (Table 9). Despite the significant time effect on phytoplankton diversity ($P < 0.05$), the post hoc Tukey test did not show significant differences among the time points. The phytoplankton diversity fluctuated between 4.4 and 5.1 genera.L^{-1} and hence the difference is negligible small (Table 9). The abundance of zooplankton was constant over time ($P > 0.05$). Zooplankton diversity was affected by time ($P < 0.05$) and the three way interaction of CS×FS×T ($P < 0.05$). The interaction effect of CS×FS×T on zooplankton diversity at each time (T) showed that the diversity of zooplankton at treatment level (CS×FS) varied only on day-14. On this day, in ponds stocked with small carps, a higher diversity of zooplankton was observed under the feeding strategy F_HS_L than under feeding strategy F_LS_H (Supplementary Figure S1).

The abundance and diversity of benthic macroinvertebrates were unaffected by the carp size and feeding strategy ($P > 0.05$; Table 9). The benthic macroinvertebrate abundance increased over time ($P < 0.001$). The benthic macroinvertebrate diversity was constant over time and not influenced by the applied treatments ($P > 0.05$; Table 9).

4. Discussion

This experiment was conducted during a warm period, with water temperatures ranging between 33°C and 35°C in the afternoon (12:00–16:00 h, as illustrated in Fig. 6). The elevated temperatures were unavoidable at the experimental site in the Khulna region, which recently frequently experiences hot days as a result of climate change (Montes et al., 2022). Having high temperature during the experiment may have had implications on survival and biomass gain of fish as temperatures often exceeded the preferred range, exposing fish to near-critical temperatures of 35°C. The optimum temperature for the cultivation of rohu is 30–33 °C (Das et al., 2005 and Ashaf-Ud-Doulah et al., 2020), for silver carp 22–28 °C (Majdoubi et al., 2022), and for both catla and tilapia 25–32 ℃ (Sharma et al., 2017; El-Sayed and Kawanna, 2008). This means that the experimental temperature exceeded the optimal cultivation temperature by up to 2°C for rohu, 7°C for silver carp, and 3°C for catla and tilapia. High mortality of small size-catla (~19 g), observed in this study (>75 %; Fig. 2 A), may have happened either because of higher sensitivity of this size class of catla to the elevated temperature or due to the lower quality of this supplied fish group or a combined result of both. Overall, for all fish species, the high temperatures possibly induced increased energy expenditure for body maintenance as reported in literature (Kordas et al., 2011; Madeira et al., 2016). This explains the observed high FCR (Table 4) because the energy gained from the feed was less utilised for biomass gain and more for body maintenance. As the whole water column (~1 m) was equally warm on high temperature days, there was no benefit for

Table 9
Main effects of carp size (CS), feeding strategy (FS) and time on plankton and benthos abundance and diversity in the pond.

Parameters	Unit	CS		FS		SEM	Time (T)				SEM	Significant effects
		Small	Big	F_LS_H	F_HS_L		D14	D28	D42	D56		
Abundance of phytoplankton	ind.L^{-1}	4562	4765	4805	4522	287	2769^c	3482^{bc}	5102^b	7300^a	443	T***
Diversity of phytoplankton	genus.L^{-1}	4.73	4.73	4.65	4.81	0.12	4.42	4.50	5.08	4.92	0.16	T*
Abundance of zooplankton	ind.L^{-1}	226	220	229	217	22	169	216	238	269	33	—
Diversity of zooplankton	genus.L^{-1}	3.19	3.08	3.02	3.25	0.19	3^b	3.58^a	3.04^{ab}	2.92^{ab}	0.20	T*, CSxFSxT*
Abundance of benthic macroinvertebrates	ind.m^{-2}	323	350	335	338	21	243^c	292^{bc}	371^{ab}	440^a	27	T***
Diversity of benthic macroinvertebrates	family.m^{-2}	4.36	4.47	4.50	4.33	0.21	4.00	4.38	4.44	4.85	0.21	—

F_LS_H = Low protein-floating feed and high protein-sinking feed; F_HS_L = High protein-floating feed and low protein-sinking feed; D = day; SEM = Standard error of mean. Parameters of phytoplankton and zooplankton were analysed by mixed ANOVA in R and of benthic macroinvertebrates were analysed by mixed model in SAS to test the effects of carp size (CS), feeding strategy (FS), time (T) and their interactions. When a significant effect was found, mean comparisons were done using Tukey's test. Per row, factor values without a letter in common are different ($P < 0.05$). P values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; Main or interaction effects that are not shown in the last column, were not significant ($P > 0.05$). For the diversity of phytoplankton, Tukey's test did not show differences among the different time points.

column/bottom feeder fish species to stay in the deeper water layers of the pond.

The experiment tested how feeding strategies and carp size affect the overall and species-specific fish performances in carp-tilapia polyculture ponds. Results indicate that the total fish productivity at pond level remained similar, regardless of the applied feeding strategies (Table 4). This is possibly because in ponds fish compensates for lower protein in one type of feed by consuming natural food as reported by Roy et al. (2023) and Roy et al. (2022). However, the similarity in total fish production in polyculture ponds between the two feeding strategies suggests that different allocation of protein and carbohydrates between the top and bottom layers of the ponds is irrelevant when the total nutrient input is the same. Feeding strategies had no influence on pond water quality and the feed intake in the pond (Tables 6 and 8). As the average feed intake time observed in this experiment was 1 h.pond^{-1} (Table 6) and there was no difference in floatability between F_L and F_H feed after one hour, floatability did not affect the feed intake of fish. The natural food web of the pond also showed no or a negligible indication of stimulation by the feeding strategies (Table 9). Silver carp, a fish species known to thrive exclusively on natural food of the pond (Cremer and Smitherman, 1980; Dong and Li, 1994), had similar production between the applied feeding strategies confirming no differences in the natural food availability between the ponds receiving different feeding strategies (Fig. 4B). This is likely because, at polyculture system level, the floating feed was eaten within an hour, which is efficient, but unfortunately, we could not observe how efficiently the sinking feed was consumed. Moreover, the short distance between top and bottom of the pond (1 m) may have masked the effect of vertical separation of the feed and thus of fed nutrients. Therefore, in deeper ponds, feeding strategies differentiating the nutrient input between pond depths might be more effective to steer pond production. Moreover, future studies should incorporate measurements of primary productivity, bacterial activity, and organic matter decomposition rates — alongside monitoring of phytoplankton, zooplankton, and benthic macroinvertebrates — to comprehensively assess in more depth the impact of feeding strategies on the pond ecosystem, than presently possible.

In this study, regardless of the feeding strategy, the productivity in ponds with small carps was higher (Table 4, Figs. 2A and 3). This was also true at species level, except for catla (Table 5 and Fig. 3), probably due to high mortality of small catla (Fig. 2 A). Although, the number of fish was higher in ponds with small carps (160) than the ponds with big carps (88), the amount of feed fed in both cases was equal. Given that, the maintenance energy cost of the higher number of small fish in ponds with small carps will be higher than in ponds with a lower number of big fish. Despite the higher maintenance cost, achieving a considerably higher biomass gain in ponds with small carps indicates that in these ponds natural food availability and their contribution to fish biomass gain was higher than the ponds with big carps. The higher production of silver carp in ponds with small carps than in ponds with big carps (Fig. 3) further supports this idea. However, question remains about how small carps affected natural food availability in the ponds. This possibly happened in two ways. First, being higher in number, fish in small carp ponds had a higher chance to eat sinking pellets and thus less feed waste accumulated in these ponds than in ponds with big carps. Second, more fish went to search feed or food at the pond bottom and thus caused more resuspension at the pond bottom which ultimately led to increased natural food production in ponds with small carps. However, the stimulating effect of carp size was not reflected in the abundance of plankton and benthos monitored (Table 9), possibly because of continuous predation pressure exerted by the fish, as observed in other studies (Kabir et al., 2019). Measuring nutrient accumulation in the sediment could provide important insights, but we did not measure it because in a previous experiment, testing different feeding strategies, while keeping the feed input in all ponds the same, did not show statistical differences between treatments. Between the ponds with small and big carps, there were negligible differences in the time and rate of intake of the floating pellets (Table 6). The effect of feeding strategy and carp size on body composition of fish was also small (Table 7, Figs. 4 and 5).

Regardless of size, the feeding strategy helped catla to mitigate the effects of high temperature. This species showed a 16 % higher survival when fed more protein at the surface of the pond under the F_HS_L feeding strategy, compared to more protein at the bottom under the F_LS_H feeding strategy (Fig. 2B). This finding aligns with existing literature, which indicates that protein supplementation mitigates the negative effect of stress in fish (Abdel-Tawwab, 2012; Kumar et al., 2011; Lieberman and Marks, 2009; Naz et al., 2023; Sarma et al., 2009). This further supports the hypothesis that surface-feeding fish benefit from a high protein-floating diet.

This study demonstrates that some fish species can withstand higher temperature better than others. Table 5 shows that although catla and silver carp experienced higher mortality, rohu and tilapia were more resilient. These findings align with Das et al. (2004) who reported catla as the least and rohu as an intermediary thermal tolerant species. The authors attributed the differences in thermal tolerance of fish to their feeding habits and argued that bottom and column feeders (mrigal (*Cirrhinus mrigala)* and rohu (*Labeo rohita*)) are more tolerant to adverse conditions than surface feeders (*Catla catla*). This likely explains the lower performance of surface feeders (catla and silver carp) compared to column/bottom feeders (rohu) and versatile feeders (tilapia) in our experiment (Table 5). These findings suggest that polyculture practices can safeguard farmers from losing their complete farming stock in the face of extreme temperatures as some species may cope while others may not. In contrast, in a monoculture system, when the cultivated fish species cannot cope with higher temperatures, farmers risk losing their fish stock and income. This study further indicates that cultivating rohu and tilapia as tolerant species can be a mitigating way for farmers to address climate change. Nevertheless, although the ponds were checked several times daily during the experiment no dead or diseased fish were observed, while the survival of catla and silver carp was lower than expected. Therefore, disease related mortality cannot be fully excluded in this study. So, more experiments should be done investigating high temperature stress in polyculture ponds, to get better insight in heat stress tolerance of different commonly cultured fish species. Considering climate change, this research is urgently needed to develop future coping strategies to maintain aquaculture productive (Mugwanya et al., 2022).

5. Conclusion

The strategy of feeding different amounts of protein and carbohydrate at different pond depths cannot influence the overall and species-specific fish production in carp-tilapia pond polyculture. Growing a higher number of small carps can considerably increase pond productivity by stimulating natural food production. Catla, especially if small in size, are highly susceptible to higher temperatures, but, feeding a high protein-floating diet improves their survival. In addition to catla, silver carp is more vulnerable to climate change compared to rohu and tilapia.

CRediT authorship contribution statement

Mohammad Mamun-Ur-Rashid: Project administration, Funding acquisition, Conceptualization. **Marc Verdegem:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Johan W. Schrama:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Uttam Adhikary:** Investigation. **Morgina Akter:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2026.103404.

Data availability

Data will be made available on request.

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