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Relationships among water quality, food resources, fish diet and fish growth in polyculture ponds: A multivariate approach

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Abstract

We examined the influence of addition of common carp (*Cyprinus carpio*) and artificial feed in rohu (*Labeo rohita*) ponds. We analyzed the relationships among four datasets on different components of the pond food web (water quality, food availability, natural food intake, and fish growth and production) with the aim to examine the effects of the addition of common carp and/or artificial feed on the different components of the pond food web, and to analyze the nature and strength of the interactions between these components. We used redundancy analysis (RDA) to investigate these effects and interactions. We found that the addition of common carp increased bio-available N and P in the water column. Artificial feed addition increased N and P only in the presence of common carp. N and P increases were more pronounced in the presence of 0.5 than in the presence of 1 common carp m^{-2}. Plankton availability was strongly positively correlated with bio-available N and P. Phytoplankton availability correlated strongest with PO_4–P, and zooplankton availability correlated strongest with PO_4–P and DO. Natural food intake in rohu was positively correlated with plankton availability in the pond water and rohu growth was also positively correlated with natural food intake. Rohu preferred plankton over artificial feed, which acted as a fertilizer for rohu growth. Common carp preferred artificial feed over natural food and its growth was higher in the presence of artificial feed and negatively correlated with natural food availability.

Keywords: Common carp; Multivariate statistics; Polyculture; Pond; Redundancy analysis; Rohu

1. Introduction

In South Asia, especially in Bangladesh, several culture combinations of indigenous and exotic carp species are commonly practiced (Wahab et al., 1994; Miah et al., 1997). The idea of polyculture is based on the principle that each species stocked has its own feeding niche that does not completely overlap with the feeding niches of other species. Therefore, a more complete use is made of the food resources and space available in polyculture than in monoculture. In some cases, one species enhances the food availability for other species and thus increases the total fish yield per unit area (Hepher et al., 1989; Miah et al., 1993; Azad et al., 2004). Traditionally in Bangladesh, 5–8 species are stocked, not always with satisfactory result, which leads to farmers using fewer species. The combination of the column-feeder rohu (*Labeo rohita*) and the bottom-feeder common carp (CC) (*Cyprinus carpio*), is rapidly becoming popular because these species realize high growth rates, while fetching excellent prices in local markets.

Since polyculture ponds are complex and not fully understood, we investigated different stocking combinations of rohu, common carp, and artificial feed addition. Read out parameters focused on four components of the pond food web, i.e. (1) water quality, (2) natural food availability, (3) dietary preference, and (4) growth and production. Although some cause-effect relationships were revealed (Rahman et al., 2006), predicting growth and production in polyculture ponds remains difficult,

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as the complex relationships between water quality, natural food types including their production and availability, fish dietary preferences, fish growth and production are not fully understood. In order to simultaneously explore the relationship between multiple variables we used multivariate statistics, which enables the analysis of overall patterns and relationships. Since we were most interested in these overall patterns and relationships between variables, rather than in formal hypothesis testing between different treatments, we chose for a graphical multivariate approach.

Multivariate techniques have been used relatively rarely for the analysis of pond aquaculture. Mostly indirect gradient analyses, such as factor analysis and principal component analysis (PCA), were used in which an ordination is calculated from one set of variables only. For examples, Milstein et al. (2002) used factor analysis to explain the relationships among different water quality variables in pond aquaculture. In another study Azim et al. (2003) used factor analysis to explore relationship between periphyton and water quality.

In this study, direct gradient analysis was used to explain the variation in one set of variables on a particular component of the food web, by a set of variables on another component. In this way, direct relationships among sets of variables related to each of the food web components were explored, which is novel in pond aquaculture. Direct gradient analysis was used as a tool to confirm established knowledge from experiments designed for testing mechanistic hypothesis (Persson and Diehl, 1990). The principal objectives of this study were to examine the effects of the addition of common carp and/or artificial feed on the different components of the pond food web, and to analyze the nature and strength of the interactions between these components.

2. Materials and methods

2.1. Pond management and experimental design

The 4.5 months experiment was carried out in 18 earthen ponds between March and July 2003 at the Field Laboratory, Faculty of Fisheries, Bangladesh Agriculture University, Bangladesh. Each of the ponds had a surface area of 100 m^2 and a depth of 1.2 m. Before the start of the experiment, ponds were drained to eradicate all weeds and animal life and repair embankments and slopes. Agricultural lime ($CaCO_3$) was applied to all ponds at 250 kg ha^{-1}. Seven days before fertilization, all ponds were individually filled with ground water from an adjacent deep tube-well. The fertilizer dose consisted of 1250 kg ha^{-1} decomposed cow manure, 31 kg ha^{-1} urea and 16 kg ha^{-1} triple super phosphate, and was applied to all ponds one week before stocking and thereafter fortnightly throughout the study period. All ponds were stocked with 1.5 rohu m^{-2}. The experiment followed a 3×2 factorial design with three levels of common carp densities (first factor; 0, 0.5 and 1.0 m^{-2}) and two levels of artificial feed (second factor; with and without feed). Each treatment had three replications. The feed contained 30% protein and was applied daily at a rate of 15 g $kg^{-0.8}$ day^{-1} from the day after releasing fingerlings until the end of the experiment. Feeding rates per pond were adjusted monthly after weighing a minimum of 30–50 fish (equalling 20% of the fishes stocked).

2.2. Water quality data

Water quality variables, viz. dissolved oxygen (DO), pH, total alkalinity, nitrate nitrogen (NO_3–N), total ammonia nitrogen (TAN), total nitrogen (TN), phosphate phosphorus (PO_4–P), total phosphorus (TP), and total suspended solids (TSS) were determined fortnightly between 9.00 and 10.00 AM. DO was measured by Winkler titration method (Stirling, 1985). The pH was measured with a Jenway model 3020 pH meter. Total alkalinity was determined by a titrimetric method (Stirling, 1985). Total ammonia nitrogen and phosphate phosphorus were analyzed with a spectrophotometer (Milton Roy Spectronic, model 1001 plus) following Stirling (1985). Nitrate nitrogen (cadmium reduction), total phosphorus (acid persulphate method) and total Kjeldahl nitrogen were determined following the methodology of APHA (1998). Total nitrogen was determined as the sum of nitrite nitrogen, nitrate nitrogen and Kjeldahl nitrogen. Total suspended solids were determined according to Stirling (1985).

2.3. Plankton and benthos data

For plankton analysis water samples were collected fortnightly by taking at 1-L sample at 10 different locations in each pond with a Niskin sampler. The composite 10-L samples were then passed through a 10-μm mesh size plankton net. Each concentrated plankton sample was transferred to a plastic bottle and diluted with formalin and distilled water to obtain 100 ml of a 5% buffered formalin solution. Plankton numbers were estimated in a Sedgewick–Rafter (S–R) cell containing 1000 fields of 1 mm^3. A 1-ml sample was put in the S–R cell and was left 10 min to allow plankton to settle. The plankton in 10 randomly selected fields in the S–R cell was identified up to genus level and counted, using the determination keys by Ward and Whipple (1959), Prescott (1962), Belcher and Swale (1976) and Bellinger (1992). Plankton density was calculated using the formula,

$$N = (P \times C \times 100)/L$$

with N=the number of plankton per liter of pond water; P=the number of planktonic organisms counted in ten fields; C=the volume of plastic bottle holding the sample (100 ml); L=the volume of the pond water sample (10 L).

The benthos samples were collected fortnightly with an Ekman dredge. In each pond, bottom mud samples from three different randomly selected sites were collected and washed through a 250 μm mesh size sieve. Benthos remaining on the sieve were preserved in a plastic vial containing a 10% buffered formalin solution. Identification keys used for benthos were after Brinkhurst (1971) and Pinder and Reiss (1983). Benthos density was calculated using the formula,

$$N = Y \times 10000/3A$$

with N=the number of benthos (m^{-2}); Y=total number of benthos counted in 3 samples; A=area of Ekman dredge (cm^2).

2.4. Diet data

Diets included all groups of phytoplankton, zooplankton and benthic macroinvertebrates, found in the gut. One fish per species per pond was collected monthly, weighed individually and killed in ice water. The body cavity was opened and the anterior five cm of the gut was removed and preserved immediately in a 10% buffered formalin solution until examined. The gut content was washed into a Petri dish and diluted to 50 ml with water. A 1-ml subsample was transferred by a pipette to an S–R cell and left for 10 min to allow the solid particles to settle. With a microscope, food items were identified up to genus and counted in 10 randomly chosen square fields of the S–R cell. The diets were quantified using the formula,

$$N = P \times C \times 100$$

with N=number of a specific food item available in the gut sample, P=total number of a specific food item observed in 10 fields, and C=volume (ml) of sample in Petri dish.

2.5. Fish growth data

Fish growth and production were estimated using average individual weight, specific growth rate (SGR) and fish yield per species. Monthly estimates were calculated. Fish were collected with a seine net and weighed to the nearest 0.1 g.

Specific growth rate (% body weight day^{-1}) was calculated using the formula (Hopkins, 1992),

$$\mathrm{SGR} = [\ln \mathrm{WT_F} - \ln \mathrm{WT_I}] \times 100/T$$

with $\mathrm{WT_F}$ = final fish weight (g), $\mathrm{WT_I}$ = initial fish weight (g), T = days between initial and final weight. Species yield was the average weight of fish multiplied by the total numbers of fish in the pond. At the end of the experiment, all ponds were drained and all fish were harvested to determine the final average weight.

2.6. Data analysis

Four different datasets were used: (1) water quality: DO, pH, total alkalinity, NO_3–N, TAN, TN, PO_4–P, TP, TSS; (2) natural foods: total Bacillariophyceae, total Chlorophyceae, total Cyanophyceae, total Euglenophyceae, total Rotifera, total Cladocera, total Copepoda and total macroinvertebrates; (3) fish diet (gut content): total Bacillariophyceae, total Chlorophyceae, total Cyanophyceae, total Euglenophyceae, total Rotifera, total Cladocera, total Copepoda and total macroinvertebrates; and (4) growth and production: average individual harvesting weight, SGR, total fish yield. Monthly mean values were calculated for all variables and for each pond, resulting in five repeated measures per pond and 90 data points in total. Percent data were arcsine transformed before analysis.

We calculated the explanatory power of each of the four datasets for all other datasets, in order to see which interactions between food web components were strongest. This means that each dataset was used both as a response and an explanatory variable in all combinations (e.g. we did not only calculate the explanatory power of water quality for diet, but also the explanatory effect of diet on water quality). We chose this approach because in complex pond interactions it is not possible to identify true 'independent' and 'dependent' variables. Our analysis resulted in 12 correlations between datasets, both for rohu and for common carp (Table 1).

Multivariate ordinations were performed with the computer program CANOCO 4 (ter Braak and Šmilauer, 1998). First, an indirect gradient analysis (detrended correspondence analysis; DCA) was executed to reveal prevailing patterns of the response variables in relation with the explanatory variable gradient (Jongman et al., 1995). Ordination axes smaller than two standard deviations indicated monotonic responses, suggesting that redundancy analysis (RDA) was the proper method for direct gradient analysis. RDA was run with variables centred and standardized by subtracting the mean and dividing by the standard deviation.

RDA was used to directly explain the variation in the response variables from the variation in the explanatory variables. To correct for the time effect, caused by the 4.5 month duration of the experiment, date was used as a covariate. The significance of first ordination axis and the significance of the first four canonical axes together were evaluated with Monte Carlo-permutation tests with 1000 permutations. Monte Carlo tests were restricted to account for repeated measures for each pond. The standard deviation of the score on the first ordination axis in the DCA was always less than 2 indicating linear or monotonic relationships. Therefore, RDA was appropriate to perform direct gradient analysis in all cases. All possible RDAs among the four datasets were performed and evaluated for the correlation between the explanatory and response variables along the first canonical axis. The highest canonical correlation indicated the highest direct explanatory power, thereby identifying the set of variables with highest explanatory power.

Table 1
Correlations among the datasets along the first canonical RDA axis (r value)

Explanatory variables	Response variables			
	Water quality	Food availability	Diet	Growth
Rohu				
Water quality	×	**0.894**	0.732	0.653
Food availability	0.710	×	**0.835**	0.742
Diet	0.535	0.459	×	**0.787**
Growth	0.588	0.092	0.703	×
Common carp				
Water quality	×	**0.894**	0.620	**0.927**
Food availability	0.710	×	**0.766**	0.839
Diet	0.473	0.525	×	0.767
Growth	0.694	0.600	0.613	×

In bold are the highest correlations for each response variable dataset.

Table 2
Redundancy analyses (RDA) of water quality explaining natural food availability

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.427	0.046	0.019	0.014
Natural food availability–water quality correlation	0.894	0.688	0.439	0.441
Cumulative % variance of natural food availability data	44.9	49.7	51.8	53.2
Cumulative % variance natural food availability–water quality relation	82.9	91.7	95.5	98.1

Total variance = 1.000; RDA was statistically significant at $P < 0.05$.

3. Results

The first canonical axis as well as the first four canonical axes combined were statistically significant at the 5% level for all RDAs. Table 1 indicates the correlations between the sets of explanatory and response variables along the first canonical axis, whereby the highest canonical correlation indicates the highest direct explanatory power. Natural food availability was best explained by water quality, and diet by natural food availability for both rohu and common carp (CC). In turn, diet best explained growth for rohu. However, CC growth was best explained by water quality. These relationships are further explored in the following sections.

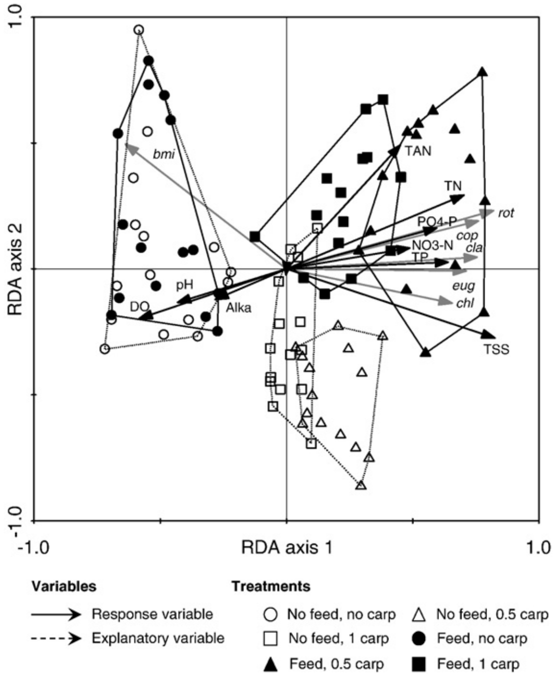

Fig. 1. RDA biplot (first two axes) of natural food availability in the pond explained by pond water quality (baci = total Bacillariophyceae, chl = total Chlorophyceae, cya = total Cyanophyceae, eug = total Euglenophyceae, rot = total Rotifera, cla = total Cladocera, cop = total Copepoda, bmi = total benthic macroinvertebrates and Alka = total alkalinity).

Table 3
Redundancy analyses (RDA) of natural food availability explaining diet of rohu and common carp (CC)

	Axis 1	Axis 2	Axis 3	Axis 4
Rohu				
Eigenvalues	0.276	0.018	0.010	0.009
Rohu diet–natural food availability correlation	0.835	0.454	0.397	364
Cumulative % variance of rohu diet data	36.4	38.8	40.1	41.3
Cumulative % variance rohu diet–natural food availability relation	86.4	92.0	95.1	97.9
Common carp				
Eigenvalues	0.124	0.015	0.010	0.006
CC diet–natural food availability correlation	0.766	0.534	0.436	0.365
Cumulative % variance of CC diet data	27.2	30.5	32.5	34.0
Cumulative % variance CC diet–natural food availability relation	76.9	86.2	92.3	96.1

Total variance = 1.000; both RDAs were statistically significant at $P < 0.05$.

3.1. Food availability explained by water quality

Two significant axes explained 49.7% of the variance in natural food availability and 91.7% of the natural food-water quality relation in the RDA (Table 2). The first RDA axis was positively correlated with the presence of CC (Fig. 1), although the lower density of CC (0.5 CC m^{-2}) obtained the highest values. This axis may be interpreted as a 'CC addition' axis, positively correlated with all nitrogenous (NO_3–N, TAN and TN) and phosphorus (PO_4–P and TP) species and all groups of phytoplankton and zooplankton availability, and negatively correlated with total alkalinity, pH and DO concentration and total benthic macroinvertebrate availability. The correlation among addition of CC, all nitrogenous and phosphorus species and all groups of phytoplankton and zooplankton availability was stronger in the presence of artificial feed than in the absence of it. The overall concentrations of nitrogenous and phosphorus species and the densities of all groups of phytoplankton and zooplankton were stronger in the treatments with 0.5 than 1 CC m^{-2}.

With the options used for RDA, a small angle between two variables is indicative of a high positive correlation between the variables, an angle of 90° indicates independence of variables, and an angle larger than 90° indicates a negative correlation. Using this way of interpretation, all groups of phytoplankton and zooplankton in the pond water were positively correlated with all nitrogen and phosphorus species and TSS, whereas negatively correlated with total alkalinity, pH and DO concentration. An opposite relation was observed in case of benthic macroinvertebrate availability. The density of benthic macroinvertebrates was positively correlated with total alkalinity, pH and DO concentration whereas negatively correlated with all other water quality parameters.

3.2. Diet explained by natural food availability

The first two canonical axes explained 38.8% of the variance in diet and 92% of the diet-natural food availability relationship in rohu and 30.5% and 86.2% of these relationships in common carp (Table 3). In rohu, the treatments with artificial feed addition scored high on the first RDA axis (Fig. 2), which may therefore be interpreted as an artificial feed addition axis. This axis is positively correlated with natural food availability and with all components of rohu diet except Euglenophyceae and Chlorophyceae ingestion. Ingestion of all groups of phytoplankton (except Euglenophyceae) and zooplankton by rohu was positively correlated with natural food availability in the water. Euglenophyceae

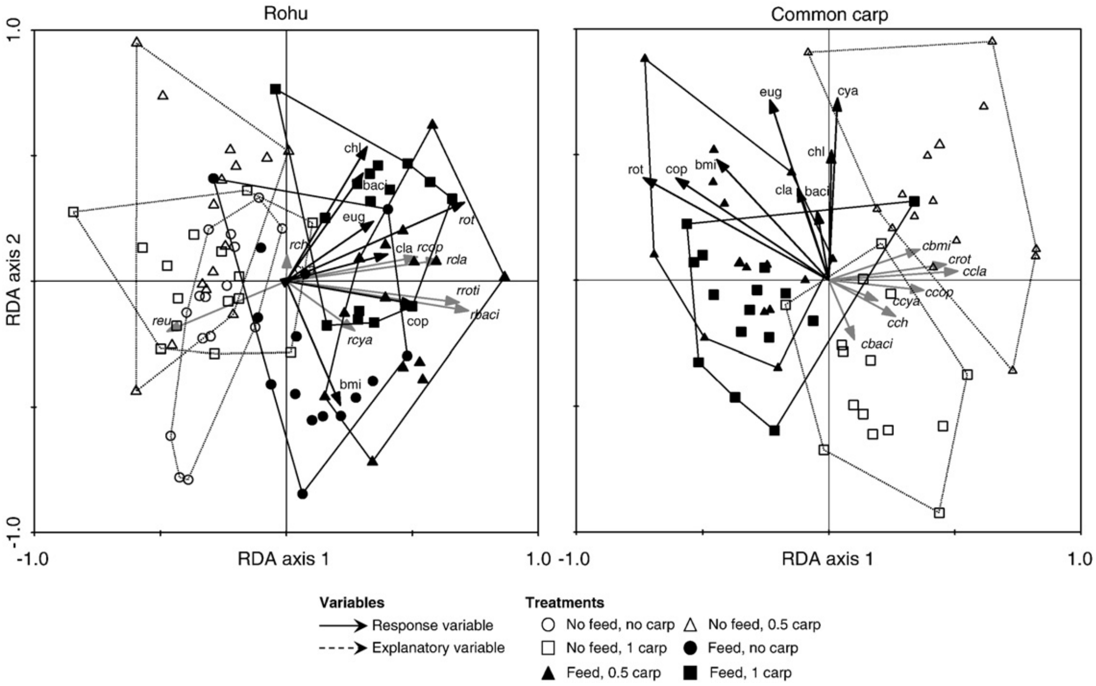

Fig. 2. RDA biplot (first two axes) of fish diet explained by food availability in the ponds water (baci = total Bacilariophyceae, chl = total Chlorophyceae, cya = total Cyanophyceae, eug = total Euglenophyceae, rot = total Rotifera, cla = total Cladocera, cop = total Copepoda and bmi = total benthic macroinvertebrate; plankton and benthic macroinvertebrate groups having d in front indicate availability of that groups in the fish diet; benthic macroinvertebrate was absent in rohu diet and Euglenophyceae was absent in CC diet).

Table 4
Redundancy analyses (RDA) of diet explaining fish growth and production of rohu and common carp (CC)

	Axis 1	Axis 2	Axis 3	Axis 4
Rohu				
Eigenvalues	0.139	0.008	0.001	0.086
Rohu growth–rohu diet correlation	0.787	0.425	0.345	0.000
Cumulative % variance of rohu growth data	51.1	53.9	54.2	55.8
Cumulative % variance rohu growth–rohu diet relation	94.4	99.6	100	
Common carp				
Eigenvalues	0.241	0.016	0.002	0.210
CC growth–CC diet correlation	0.767	0.394	0.180	000
Cumulative % variance of CC growth data	41.2	44.0	44.4	80.3
Cumulative % variance CC growth–CC diet relation	92.8	99.1	100	

Total variance = 1.000; both RDAs were statistically significant at $P < 0.05$.

ingestion was negatively correlated with natural food availability in the water. The additional effect of CC on natural food availability and rohu diet was only slight. The highest levels of natural food availability and ingestion were found in the presence of 0.5 CC m^{-2}, followed by 1 and 0 CC m^{-2}, but there was a large overlap.

In case of CC, we observed almost the opposite to the situation with rohu. Here the treatments with the addition of artificial feed scored low on the first RDA axis. Natural food ingestion was lower in the presence of artificial feed, even though natural food availability (especially zooplankton and benthic macroinvertebrates) was higher (Fig. 2). In the absence of artificial feed, the treatment with 0.5 CC m^{-2} scored higher on the second RDA axis than the treatment with 1 CC m^{-2}. In case of the lower CC density, the availability of all natural foods was higher, but there appeared not to be a clear relationship with diet. Only the amount of ingested Bacillariophyceae was higher in case of the higher CC density.

3.3. Fish growth explained by fish diet

The first two canonical axes explained 53.9% of the variance in growth and 99.6% of the growth-natural food availability relationship in rohu and 44.0% and 99.1% of these relationships in common carp, respectively (Table 4). In rohu, the treatments with artificial feed addition scored high on the first RDA axis (Fig. 3), which may therefore be interpreted as an artificial feed addition axis. This axis is positively correlated with rohu diet (except for the ingestion of Euglenophyceae) and growth variables. Rohu growth variables were strongly positively correlated with all rohu's phytoplankton (except Euglenophyceae) and zooplankton ingestion variables.

In case of CC the treatments with artificial feed addition scored low on the first RDA axis (Fig. 3) and the growth variables had a strong negative correlation with the first RDA axis. This indicates that CC's growth variables were negatively correlated with natural food ingestion. In case of 0.5 CC m^{-2}, harvesting weight and growth rate were on average higher than in the case of 1 CC m^{-2}. This corresponded with a higher ingestion of benthic macroinvertebrates, copepods and rotifers, and a lower ingestion of phytoplankton. The effect of zooplankton and benthic macroinvertebrate ingestion on overall ordination was higher than that of phytoplankton ingestion.

3.4. Fish growth explained by water quality

The first two canonical axes explained 46.0% of the variance in growth and 99.1% of the growth-water quality relationship in rohu and 73.1% and 92.6% of these relationships in common carp (Table 5). In rohu, the treatments with CC addition scored higher on the first RDA

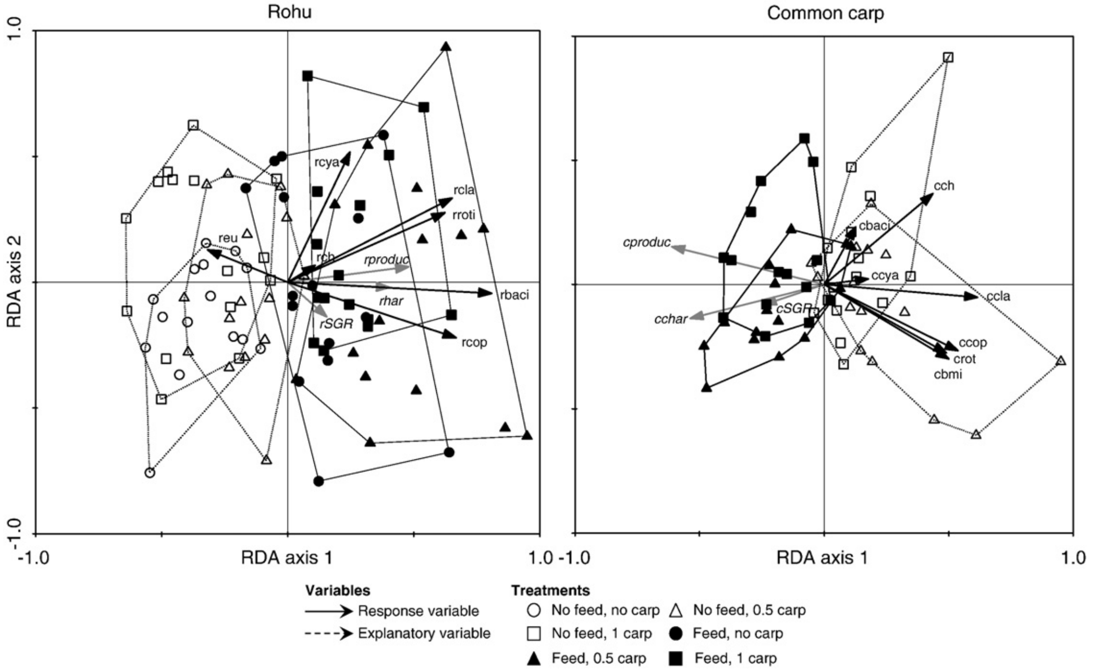

Fig. 3. RDA biplot (first two axes) of growth explained by fish diet (dbaci = total Bacillariophyceae, dchl = total Chlorophyceae, dcya = total Cyanophyceae, deug = total Euglenophyceae, drot = total Rotifera, dcla = total Cladocera, dcop = total Copepoda, dbmi = total benthic macroinvertebrate, har = average harvesting weight of fish and produc = total production of fish).

Table 5
Redundancy analyses (RDA) of water quality explaining fish growth and production of rohu and common carp (CC)

	Axis 1	Axis 2	Axis 3	Axis 4
Rohu				
Eigenvalues	0.095	0.030	0.001	0.130
Rohu growth–water quality correlation	0.653	0.821	0.546	0.000
Cumulative % variance of rohu growth data	35.1	46.0	54.6	94.1
Cumulative % variance rohu growth–water quality relation	75.5	99.1	100	
Common carp				
Eigenvalues	0.364	0.063	0.034	0.066
CC growth–water quality correlation	0.927	0.787	0.759	0.000
Cumulative % variance of CC growth data	62.2	73.1	78.9	90.3
Cumulative % variance CC growth–water quality relation	78.9	92.6	100	

Total variance = 1.000; both RDAs were statistically significant at $P < 0.05$.

axis (Fig. 4) than those without CC addition. This axis is positively correlated with most water quality variables (except for alkalinity, pH and DO) and all growth variables. In the presence of CC, the addition of artificial feed resulted in higher RDA scores. This effect was stronger in the case of 0.5 CC m^{-2} than in the case of 1 CC m^{-2}.

In case of CC, the first RDA axis was positively correlated with artificial feed addition and may be interpreted as a feed addition axis. As in the case of rohu, this axis was positively correlated with all growth variables and most of the water quality variables, except for pH, DO, and alkalinity. In the presence of artificial feed, this effect was stronger in the cases with 0.5 CC m^{-2} than in those with 1 CC m^{-2}. In the treatments with 0.5 CC m^{-2}, CC also scored higher on the second RDA axis, correlating with harvest weight and growth rate of CC, and with all water quality variables, except for TAN and NO_3–N.

4. Discussion

This study shows the overall patterns of the effects of feed addition and the addition of CC on water quality and natural food availability in rohu ponds and on the diet, and growth of rohu and CC in these ponds. The water quality dataset explained the overall variation in natural food availability quite well (correlation along the first RDA axis was 0.894). Moreover, the variation of water quality and natural food availability was related to the addition of CC and its density (Fig. 1). These observations are somewhat in concordance with Milstein et al. (2002), Parkos et al. (2003) and Ritvo et al. (2004), who mentioned changes of water quality with addition of CC. However, the lower density of CC (0.5 CC m^{-2}) resulted in stronger effects on water quality than the higher density (1 CC m^{-2}). This might be caused by the fact that when CC is present at a higher density the grazing pressure on natural food is higher, resulting in lower densities of natural food. The lower biomass of natural food released less available N- and P-species in the water column (Kibria et al., 1997; Attayde and Hansson, 1999), resulting in an overall lower concentration of N and P in treatments with 1 than 0.5 CC m^{-2}. The addition of artificial feed further increased the effects of CC on water quality and natural food availability, while it had no effect in the absence of CC (Fig. 1). The higher amounts of N and P were correlated with higher densities of phytoplankton and zooplankton. In contrast they were negatively correlated with dissolved oxygen, pH and alkalinity. This can be explained by the higher oxygen consumption and carbon dioxide production during decomposition of organic material (Moriarty, 1997). Higher concentrations of carbon dioxide result in a lower pH and alkalinity. Artificial feed supplied additional nutrients and CC

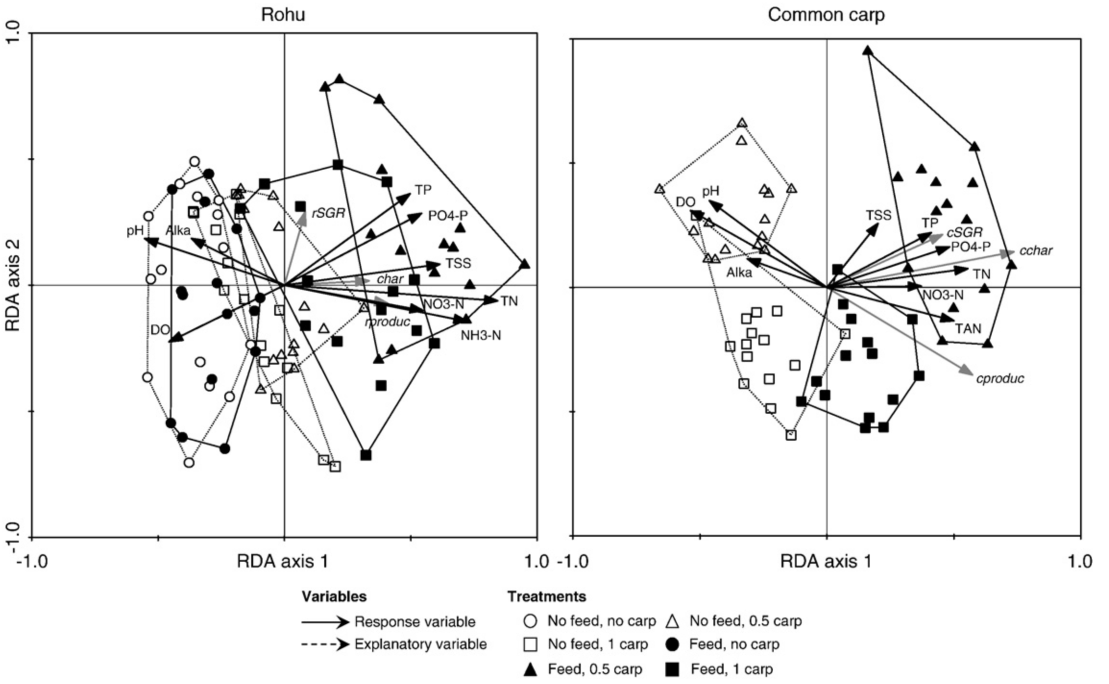

Fig. 4. RDA biplot (first two axes) of fish growth explained by pond water quality (har = average harvesting weight of fish, produc = total production of fish and Alka = total alkalinity).

increased decomposition and liberation of those nutrients from sediment to the water column (Hohener and Gachter, 1994), resulting in the observed patterns.

It was found that PO_4–P and NO_3–N were strongly positively correlated with phytoplankton and zooplankton biomass. PO_4–P had the strongest overall correlation with phytoplankton density (longer vector in Fig. 1) and with natural food availability in general. The correlation between PO_4–P and total phytoplankton biomass was stronger in treatments without CC ($r=0.64$, $P<0.01$) than with CC ($r=0.50$, $P<0.01$), which might indicate that phytoplankton biomass was limited by PO_4–P concentrations in treatment without CC. This is in accordance with Schindler (1988), Elser et al. (1990) and Diana et al. (1997), who stated that phosphorus is a major limiting nutrient in most freshwater ecosystems. In another study, Smith (1985) showed that phytoplankton production at optimum light intensity was highly dependent on phosphorus. Overall zooplankton densities are best explained by PO_4–P and DO concentrations. Total zooplankton biomass was strongly correlated with these two factors (PO_4–P: $r=0.68$, $P<0.01$; DO: $r=-0.65$, $P<0.01$) whereas correlations with all other water quality parameters were weak. The positive correlation between zooplankton and PO_4–P might be caused directly through the release of PO_4–P by zooplankton (Wen et al., 1994; Ikeda et al., 1982), and indirectly via phytoplankton production. Some zooplankton species are known not to tolerate low oxygen concentrations (Elgmork, 1959). However, DO concentration generally affects zooplankton at much lower concentrations (<2.5 mg L^{-1}: Hanazato et al., 1989; Bertilsson et al., 1995) than found in this study (range: 4.6–7.7 mg L^{-1}) and therefore it is not likely that it played a significant role in structuring the zooplankton community in the ponds. The negative correlation between DO concentration and zooplankton density was most probably caused by, (i) the respiration of zooplankton, and (ii) decomposition of organic matter produced by the zooplankton. A similar negative correlation between zooplankton availability and DO concentration was documented by Aka et al. (2000) and Dresilign (2003).

We found that for rohu overall diet was best explained by natural food availability (correlation along the first RDA axis: $r=0.835$; Table 1). These results clearly indicate that rohu mainly feeds on natural food and that the positive effect of artificial feed on natural food availability, ingestion and growth is indirect. Probably the artificial feed acts as a fertilizer (Dewan et al., 1988; Diana et al., 1997), increasing the amount of natural food through the higher concentrations of nutrients in presence of common carp (Fig. 1). This allowed rohu to eat higher amounts of phytoplankton and zooplankton, which is its preferred food when foraging in the water column (Dewan et al., 1977; Jhingran and Pullin, 1985; Wahab et al., 1994). Therefore, addition of artificial feed and common carp both are important factors for rohu's natural food ingestion and growth and production.

The effects of ingestion of all zooplankton groups on the overall ordination are stronger than the effects of almost all phytoplankton ingestion variables (except for Bacillariophyceae; Fig. 2)). These results indicate that rohu reacts more strongly on changes in zooplankton availability, suggesting that rohu prefers zooplankton over phytoplankton. This result somewhat agrees with Miah et al. (1984), who mentioned rohu fry prefer zooplankton above phytoplankton.

In CC, we found that the overall diet was best explained by food availability (correlation along the first RDA axis: $r=0.766$; Table 1), but not as good as in rohu ($r=0.835$). The overall relationship between the natural food ingestion and growth and production datasets in CC is lower ($r=0.767$ along the first RDA axis; Table 1), than the relationship between water quality and growth and production datasets ($r=0.927$ along the first RDA axis; Table 1), probably because artificial feed ingestion was not used as a variable in the analysis. In CC, ingestion of natural food was lower and growth and production was higher with addition of artificial feed, although availability of natural food was higher with addition of artificial feed (Figs. 2, 3). These results indicate that common carp prefers artificial feed over natural foods. In absence of artificial feed, CC preferred zooplankton and benthic macroinvertebrates when natural food was relatively more abundant in presence of 0.5 CC m^{-2}, but it switched to ingest phytoplankton when natural food was less abundant in presence of 1 CC m^{-2} (Fig. 2). This result somewhat agrees with Spataru et al. (1983), Sibbing (1988) and Garcia-Berthou (2001), who mentioned CC is a bottom feeder, feeding on zooplankton and benthic macroinvertebrates but it can also feed on phytoplankton. The strongest correlation between water quality and growth and production of CC might be related with stirring effects, which increased nutrient fluxes from the sediment to the water column (Graneli, 1979; Hohener and Gachter, 1994; Hargreaves, 1998).

Our results show that in the management of rohu ponds addition of artificial feed and stocking of CC have positive, but interacting, effects on total pond yield. The addition of feed appears to be most profitable in the presence of an intermediate density of CC, because then CC can profit most of both natural food (especially macroinvertebrates) and artificial feed, while, at the same time, rohu profits most from the fertilizing effect of feed addition and the stirring effect of CC. At higher densities of CC competition between CC for the available macroinvertebrates may be stronger (Rahman et al., 2006). This could lead to a more efficient uptake of artificial feed by CC, leaving less feed available for pond fertilization. This would result in less plankton production, which is the preferred food of rohu. The existence of an intermediate optimum stocking density of CC to increase total fish yield could be taken into account by pond managers, for instance by implementing small-scale trials with different stocking densities.

5. Conclusions

For rohu and common carp in polyculture ponds we found that water quality variables were the best explanatory variables for natural food availability, which in turn explained natural food ingestion best. In the case of rohu, natural food ingestion explained most of the variation in growth and production, but not for common carp, which preferred artificial feed over natural food. The stepwise correlation of growth and production of rohu and carp from datasets of water quality, food availability and food ingestion, using redundancy analysis explained more of the total variation than would have been shown by separately correlating these datasets. Therefore we believe that this approach could be a promising tool for elucidating causal links in pond aquaculture research.

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