Food Web Interactions and Nutrients Dynamics in Polyculture Ponds

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Dedicated to my wife

Contents

Chapter 1

General Introduction

Potentialities of aquaculture in Bangladesh

As the world population continues to increase, demand for fish products has increased beyond total supply, resulting in a reduction of the global per capita capture fisheries supply. To fill the gap, aquaculture boomed. Currently, aquaculture is the fastest growing animal production sector in the world expanding at an average of 9.2% per year since 1970 compared to only 1.4% for captured fisheries and 2.8% for terrestrial animal meat products (FAO, 2002).

Fish dominates the diet of the people of Bangladesh to such an extent that the old proverb “mache bhate Bangali” which can be translated as “fish and rice make a Bangali”, remains true. Rice, fish and a little vegetable are the ingredients of a typical Bangali meal. Historically, the people of Bangladesh depended mainly on capture fisheries to obtain fish. Overfishing and environmental degradation caused catches to decline and people began to culture fish in enclosed waterbodies like ponds or ditches. At present, aquaculture provides close to 50% of the total inland fish production. Over the last two decades, inland freshwater aquaculture production grew more than 10% annually, relying mainly on polyculture of native and exotic carp species in ponds (DOF, 2005).

Bangladesh is one of the world’s leading fish producing countries. In 2003, it was the sixth largest aquaculture producing country in the world, supplying 12% of global aquaculture production, excluding China (FAO, 2005). The country is situated in the multiple-delta of the rivers Brahmaputra, Meghna and Ganges. It has an extensive inland water resource comprising 265,500 ha of ponds (total 1.3 million ponds) and ditches, 5,488 ha of oxbow lakes, 141,353 ha of shrimp farms, 1,031,563 ha of rivers and estuaries, 114,161 ha of beels (shallow natural depression), 68,800 ha of man-made reservoirs (Kaptai lake) and 2,832,792 ha of floodplains. The total fish production in 2003 was about 2 million metric tones (MT) of which 43% came from aquaculture (FAO, 2005); pond aquaculture contributed 89% to the total aquaculture (DOF, 2005).

At national level, the fisheries sector in Bangladesh contributes significantly to nutrition, employment, household income, and foreign exchange earnings. Fish provides 63% of the animal protein intake in Bangladesh. About 1.3 million people rely fully and another 12 million people rely partly on this sector. Fish and fishery products are the country's second largest export commodity contributing about 10% of annual export earning and about 5.2% of national GDP, or 20% of the agriculture GDP (DOF, 2003; Shah, 2003).

In Bangladesh aquaculture has developed mainly as a rural activity integrated into existing farming systems. Aquaculture could play a more important role as a stimulant for the economic activity, especially in the rural areas. It has a great potential to be a major income-generating component to poverty alleviation. Introducing a developed sustainable rural pond fish farming system can improve the economic sustainability of poor farmers, who still provide the bulk of the production in south Asia (Sinha and Saha, 1980; ADB, 2004).

The present per capita annual fish consumption in Bangladesh stands at about 14 kg yr^{-1} against a recommended minimum requirement of 18 kg yr^{-1}. Prospects to supply protein by increasing livestock production are not bright, because there is an increased competition to grow food grains for human consumption instead of livestock. Therefore, the only way to increase the supply of animal protein is through increasing aquaculture productivity. The average production is still low: 2,609 kg ha^{-1} yr^{-1} for ponds and ditches, 780 kg ha^{-1} yr^{-1} for oxbow lakes and 565 kg ha^{-1} yr^{-1} for coastal aquaculture. However, the potentials are much higher.

Status of carp polyculture

The principal cultured carp species by value in the world are bighead carp (*Aristichthys nobilis*), black carp (*Mylopharyngodon piceus*), catla (*Catla catla*), common carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*), grass carp (*Ctenopharyngodon idella*), mrigal (*Cirrhinus cirrhosus*), rohu (*Labeo rohita*) and silver carp (*Hypophthalmichthys molitrix*). Carps contribute more than 70% to the inland aquaculture production in Bangladesh, south Asia, Asia and the World (Table 1) and are thus the major provider of fish protein through aquaculture (Acosta and Gupta, 2005). During the last decade carp culture in Asia grew on average 12% annually (Dey et al., 2005). In south Asia alone, carp production increased from 1.43 million MT in 1995 to 2.53 million MT in 2003 (Table 1).

Ponds are the principal aquaculture production system in Bangladesh, producing on average 85 and 86% of total aquaculture and carp production, respectively (Alam, 2002; DOF, 2003). Although covering much larger areas than ponds, the total fish production from rivers and estuaries, beels, lakes, floodplains, ditches and oxbow lakes is considerably smaller (Sharma and Leung, 2000; Ayyappan, 2003). Generally, semi-intensive polyculture with various carp species is practiced in ponds (Miah et al., 1997; Wahab et al., 1994; Reddy et al., 2002). A combination of compatible species with complementary feeding habits is usually stocked to make better use of the natural food available (Kumar, 1992). Resource-poor farmers prefer semi-intensive carp polyculture because the capital needed to buy (expensive) artificial feeds is minimized, while the exploitation of natural foods in ponds is optimized. Moreover, semi-intensive polyculture is especially suitable for most of the carp species because of their higher preference for natural food in comparison to artificial feed. Nevertheless, there is a tendency by richer farmers to further increase production through the application of artificial feeds.

The indigenous major carp species catla, mrigal and rohu are the most important culture species in south Asia (Uddin et al., 1994; FAO, 1997; Kanak et al., 1999). In most cases, farmers raise these three species in combination with two or more exotic species, such as common carp, silver carp, Thai sarpunti (*Puntius gonionotus*)**,** and grass carp. Often they are disappointed with slow growth and low production and complain about the complexity of the technology. The feeding niches of some carp species in polyculture are reasonably well known, but predicting synergism or competition between species remains difficult. A better understanding of intra- and inter-species effects on natural food availability, its exploitation and fish production is needed for optimization of existing culture practices.

Of all species stocked in polyculture, rohu is the most important one, contributing on average 23% of the total aquaculture production in the region (Figure 1). Fish farmers in south Asia like to stock rohu as their main aquaculture species, because it fetches a favourable price and has a high consumer preference (Dey et al., 2005). Rohu is known to feed mainly in the water column (Dewan et al., 1977; Jhingran and Pullin, 1985). To make better use of the natural food available in the pond, a benthic feeder like mrigal was traditionally stocked alongside. As the production systems are continuously changing, nowadays, farmers prefer to stock common carp as a benthic feeder instead of mrigal, because common carp grows faster than mrigal and the overall production is higher when combined with other native major carps in polyculture ponds (Dewan et al., 1985; Wahab et al., 1995; Milstein et al., 2002). Still, little is known on how common carp and rohu interact in the exploitation of natural foods in ponds or on how they influence natural food availability.

Table 1. Carp production (×10^3 MT) from aquaculture of the Bangladesh, south Asia, Asia and the World.

Year	Bangladesh	South Asia*	Asia	World
1995	198 (73, 63)	1,434 (76, 71)	10,133 (80, 36)	10,395 (77, 33)
1996	239 (76, 63)	1,723 (85,79)	11,714 (82, 38)	11,982 (78, 35)
1997	340 (83, 70)	1,927 (86, 81)	12,943 (81, 40)	13,209 (78, 37)
1998	402 (82, 70)	1,984 (84, 79)	13,618 (81, 38)	13,892 (77, 36)
1999	416 (82, 70)	2,165 (83, 78)	14,584 (80, 37)	14,897 (77, 35)
2000	459 (80, 70)	2,190 (90, 83)	15,076 (78, 36)	15,385 (75, 34)
2001	512 (81, 72)	2,433 (91, 85)	15,877 (78, 36)	16,203 (75, 33)
2002	567 (81, 72)	2,465 (88, 82)	16,274 (75, 36)	16,599 (72, 32)
2003	617 (80, 72)	2,529 (87, 81)	16,798 (73, 34)	17,127 (71, 31)

First and second values within parenthesis indicate carp production as percentage of freshwater aquaculture and total aquaculture production, respectively.

*Includes Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka.

(Reproduced from FAO, 2005)

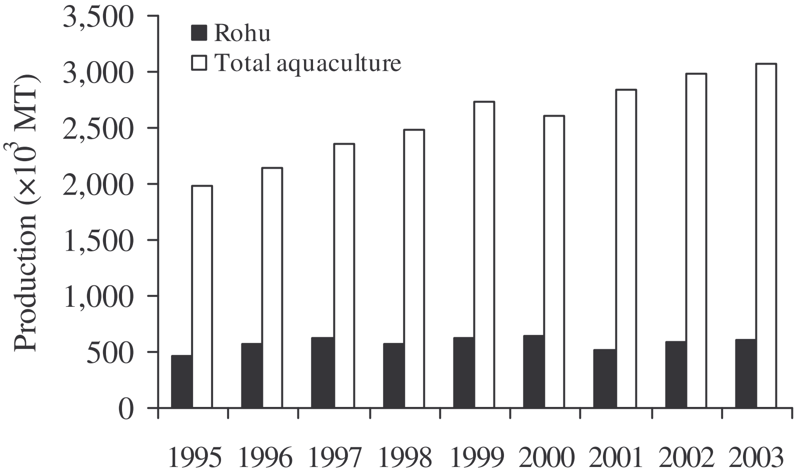

Figure 1. Rohu and total aquaculture production in Bangladesh and India (reproduced from FAO, 2005).

Benthivorous fish and their role in productivity

A relatively large number of benthivorous carp species are cultured including common carp (Spataru et al., 1980; Hepher and Pruginin, 1981; Spataru et al., 1983), crucian carp (Prejs, 1976; Holopainen and Hyvarinen, 1985), mrigal (Milstein et al., 2002), kalibaus *Labeo calbasu* (Milstein et al., 2003), bream *Abramis brama* (Kennedy and Fitzmaurice, 1968; Lammens et al., 1987; Meijer et al., 1990), white bream *Blicca bjoerkna* (Lammens, 1984; Brabrand, 1984; Rask, 1989), barb *Barbus barbus* (Granado-Lorencio and Garcio-Novo, 1986), gudgeon *Gobio gobio* (Kennedy and Fitzmaurice, 1972) and tench *Tinca tinca* (Kennedy and Fitzmaurice, 1970). Most of these are specialized to feed on benthic organisms and in doing so affect water transparency, nutrient cycling, and phytoplankton, zooplankton, macrophyte, and benthic macroinvertebrate abundances (Northcote, 1988). Chironomid larvae are one of the important benthic food sources. Depending on their size, species and sediment type, chironomid larvae dwell from a few millimetres to several centimetres deep in the sediment (Winkel, 1987). Therefore, fish predation on chironomids requires specialized techniques to separate them from the sediments (Hyslop, 1982; Robotham, 1982; Sibbing, 1988). By digging and sieving of sediments, benthivorous fishes increase oxygen availability in the sediment and cause resuspension of bottom particles, which in turn has a large impact on the abiotic and biotic properties of the overlying water column.

The mineralization of organic matter happens faster under aerobic than under anaerobic conditions. Beristain (2005) showed that the mineralization rate of aquaculture feeds in laboratory scale 2 L microcosms was around 4 times faster under aerobic than under anaerobic conditions. Therefore, favouring aerobic decomposition of organic matter will stimulate nutrient cycling in ponds. The digging and sieving of sediments by benthivorous fish also increased diffusion rates across the sediment-water interface (Hohener and Gachter, 1994), which in turn increase nutrient availability in the overlying water column. If not excessive, an increase in nutrient availability will enhance primary production, which will then enhance secondary and tertiary production. However, if excessive, negative effects will occur, such as:

(i) an increase of grazing pressure on natural food specially zooplankton and benthic macroinvertebrates to such an extent that further recovery is not possible,

(ii) an increase of the redox potential through increased oxygen supply, at redox potentials above 200 mV, a portion of soluble phosphorus (PO_4-P) will precipitate, forming

Table 2. An overview of the study on the effects of benthivorous fish on biotic and abiotic components in ponds and lakes.

Main effect	References
Macrophytes	Brumley, 1991; Fletcher et al., 1985; Hinojosa-Garro and Zambrano, 2004; King and Hunt, 1967; Laugheed and Chow-Fraser, 2001; Lougheed et al., 1998; McCrimmon, 1968; Miller and Crowl, 2006; Parkos III et al., 2003; Roberts et al., 1995; Sidorkewicj et al., 1996; Zambrano and Hinojosa, 1999.
Periphyton	Sidorkewicj et al., 1999.
Phytoplankton	Beklioglu et al., 2003.
Zooplankton	Laugheed and Chow-Fraser, 2001; Parkos III et al., 2003.
Benthic community	Barton et al., 2000; Miller and Crowl, 2006; Parkos III et al., 2003; Riera et al., 1991; Tatrai et al., 1994; Wilcox and Hornbach, 1991; Zambrano and Hinojosa, 1999; Zur, 1979.
Turbidity	Barton et al., 2000; Beklioglu et al., 2003; Chow-Fraser, 1999; Fernandez et al., 1998; Fletcher et al., 1985; Laugheed and Chow-Fraser, 2001; Lougheed et al., 1998; Meijer et al., 1990; Parkos III et al., 2003; Sidorkewicj et al., 1996; Tatrai et al., 1997.
Status of sediment N and P	Jana and Chakrabarty, 1997.
Water P	Karjalainen et al., 1999; Parkos III et al., 2003.
Water N	Karjalainen et al., 1999.
Bioturbation/resuspension	Breukelaar et al., 1994; Ritvo et al., 2004; Tatrai et al., 1997.
Comparison between two benthivorous on environment	Milstein et al., 2002.
Comparison between two benthivorous on fish production	Wahab et al., 2002.

inorganic particles (e.g., with iron as iron (III) phosphate) (Holdren and Armstrong, 1980; Bostrom et al., 1988; Boyd, 1995), and

(iii) a higher turbidity due to resuspended particles, which reduce light penetration and hence photosynthesis.

Considering both positive and negative effects, stocking densities of benthivorous fishes are important. A considerable amount of work has been done on the effects of benthivorous fish on biotic and abiotic components in ponds and lakes (Table 2). Most of these studies stressed the effects of benthivorous fish on vegetation, benthic communities and turbidity. Very little attention has been paid on increasing fish production by inclusion of benthivorous fish. In pond aquaculture, little is known about the combined effects of benthivorous and planktivorous species on food availability, fish production, water quality, nutrients retention in natural foods and fish, and fish behaviour.

Role of artificial feed in productivity

In aquaculture especially in semi-intensive systems, artificial feed has two main functions, (i) to be directly eaten by fish, and (ii) to supply nutrients to the environment, which in turn increases natural food availability (Figure 2). The major portion (≈ 80%) of artificial feed is lost in the system as uneaten feed and faeces (Daniels and Boyd, 1989; Siddique and Al-Harbi, 1999). Artificial feed, which is lost in the system, has a great effect on water quality through decomposition (Horner et al., 1987; Poxton and Allouse, 1987; Poxton and Lloyd, 1989). Feed contains starch and proteins; having a first order decomposition rate of about 0.8 day^{-1} (van Keulen and Seligman, 1987). This increases bioavailable nitrogenous and phosphorus compounds and CO_2 concentration and decreases the dissolved oxygen (DO) concentration and pH, resulting in lower alkalinity values of the water. Bioavailable nitrogenous and phosphorus compounds along with CO_2 enhance photosynthesis and in turn the production of natural food. The availability of natural food may change the food habits, grazing, behaviour and growth of fish. However, the underlying mechanisms remain unclear.

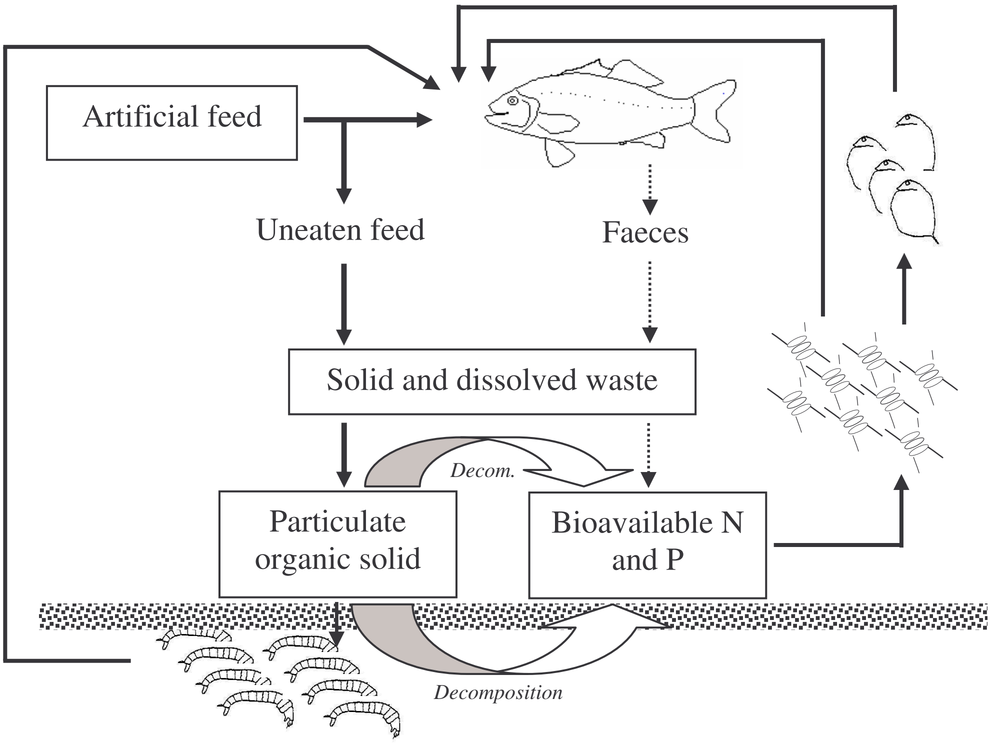

Figure 2. Schematic diagram of the role of artificial feed in fish pond.

The choice for rohu and common carp

For this study we wanted to choose a combination of a planktivorous and a benthic feeding fish species. We chose for rohu and common carp, because they are important commercial aquaculture fish species. In 2003, these two species combined contributed 26% (common carp 15%, rohu 11%) of the total freshwater aquaculture production in the world, except China (FAO, 2005). In addition, rohu was chosen as the planktivorous fish species because it can exploit food from the whole water column (Das and Moitra, 1956) as well as having high consumer preference and market value (as mentioned before) in south Asia especially in Bangladesh and India (Dey et al., 2005). Common carp was chosen as the benthivorous fish species because behaviour of this species is mostly known in aquatic ecosystem. Moreover, it is found in almost all the countries of the world, and farmers in Bangladesh prefer common carp above any other benthivorous species due to its fast growth (Wahab et al., 1995; Milstein et al., 2002; Zambrano et al., 2001).

Hypothesis, objectives and overview of the thesis

The overall objective of this thesis is to determine the effects of different densities of common carp in polyculture with rohu on the pond ecosystem, nutrient dynamics and productivity. It is hypothesised that common carp density affects nutrient resuspension, water quality, availability of natural food, fish behaviour and production in rohu-common carp polyculture ponds. Because richer farmers sometimes apply artificial feeds, the effects of artificial feed application were also assessed.

This PhD thesis starts with a general introduction (this Chapter) and concludes with a general discussion (Chapter 6). The remaining Chapters focus on different aspects of limnological changes, fish growth and behavioural responses under different common carp densities in fed or non-fed rohu ponds. All possible treatment combinations with two major factors (i) common carp density (3 levels: 0, 0.5 and 1 common carp m^{-2}) and (ii) artificial feed addition (2 levels: with or without artificial feed) were tested. Attention was also paid to the interaction between common carp density and artificial feed. In Chapter 2, all results obtained from a pond study were analysed with multivariate techniques. Datasets on water quality, natural food availability, natural food ingestion, and growth and production were analysed for their mutual relationships. Chapter 3 elucidates the effects of artificial feed and common carp density on natural food availability, food preference, food intake, growth and production in semi-intensively managed rohu ponds. It was found that food availability was lower at a density of 1 than at 0.5 common carp m^{-2}. Therefore, in Chapter 4 the underlying mechanisms of nutrient availability were related to (i) fish species-water quality interactions and (ii) nutrient retention efficiency by fish and the various types of natural foods available in the pond, including phytoplankton, zooplankton and benthic organisms. Chapter 2, 3 and 4 suggested synergistic effects between rohu and common carp on production. The question was raised if the observed synergism was mainly a matter of food availability or also of changes in feeding behaviour. In Chapter 5 changes in fish behaviour of different stocking combinations of common carp and rohu were analyzed. Chapter 6 integrates and interprets the major conclusions of the previous Chapters, discusses consequences for farming practices, reflects on the limitation of the experimental setup used and gives suggestions for further studies.

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Chapter 2

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 (water quality, food availability, natural food intake, and fish growth and production) with the aims (1) to obtain an integrated view on the effects on the pond system of the addition of common carp and/or artificial feed, (2) to investigate/confirm the mechanisms in which different components of the pond food web interact, and (3) to investigate the strength of the links between different sets of food web variables in pond aquaculture. We applied a multivariate approach, using redundancy analysis (RDA). We found that both the addition of common carp increased bioavailable N and P in the water column and that artificial feed addition increased this effect only in presence of common carp. The effects were more pronounced in the presence of 0.5 than in the presence of 1 common carp m^{-2}. Plankton availability in the pond water was strongly positively correlated with bioavailable 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. The multivariate approach was useful for investigating the direct relationships between datasets, which was helpful in investigating the causal links in the pond ecosystem.

Keywords: Multivariate statistics; Polyculture; Pond; Rohu; Common carp; Artificial feed.

INTRODUCTION

In south Asia, especially in Bangladesh, several culture combinations of indigenous and exotic carp species are commonly practiced (Miah et al., 1997; Wahab et al., 1994). 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, 5-8 species were stocked be it not always with satisfactory results, and nowadays farmers have a tendency to use fewer species. Especially the combination of the column-feeder rohu (*Labeo rohita*) and the bottom-feeder common carp (CC) (*Cyprinus carpio*), is rapidly becoming more popular because these species realize high growth rates while fetching excellent prices in local markets

Since polyculture ponds are complex, not fully understood, ecological systems the effects of different stocking combinations of rohu and common carp and artificial feed addition were investigated. Read out parameters focussed on natural food availability, dietary preference, water quality and growth and production. Although some cause-effect relationships were revealed (Rahman et al., 2006), predicting growth and production in polyculture ponds remains difficult, 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 a multivariate statistics, which enables the analysis of overall patterns and relationships. 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 by a second set of variables. In this way, direct relationships among sets of variables related to water quality, natural foods abundance, dietary preferences, and fish growth and production 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 (1) to obtain an integrated view on the effects of adding common carp and/or artificial feed on pond functioning, (2) to analyze the mechanisms by which different components of the pond ecosystem interact, and (3) to investigate the strength of the links between different sets of variables in pond aquaculture.

MATERIALS AND METHODS

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 the 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 1,250 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 minimum 20% of the fishes stocked (30-50 fish).

Water quality data

Water quality parameters, 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).

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 minutes 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 litre 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. Per 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 was preserved in a plastic vial containing a 10% buffered formalin solution. Identification keys used for benthos were 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).

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 sub-sample was transferred by a pipette to an S-R cell and left for 10 minutes to allow the solid particles to settle. With a microscope food items were identified up to genus level 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 = no. of a specific food item available in the gut sample, P = total no. of a specific food item observed in 10 fields, and C = volume (ml) of sample in Petri dish.

Fish growth data

The fish growth and production related parameters average individual weight, specific growth rate (SGR) and fish yield per species were determined monthly. Fish were collected by a seine net and weighed on a balance with a precision of 0.1g. Specific growth rate (% body weight day^{-1}) was calculated using the formula,

$$SGR = [\ln WT_F - \ln WT_I] \times 100/T$$ (Hopkins, 1992)

with WT_F = final fish weight (g), WT_I = initial fish weight (g), T = days between initial and final weight. Species yield was calculated as average weight of fish multiplied by 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.

Data analysis

Four different data sets 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. Percent data were arcsine transformed before analysis. Multivariate ordinations were performed with the computer program CANOCO 4 (ter Braak and Smilauer, 1998). First, an indirect gradient analysis (detrended correspondence analysis; DCA) was executed to reveal prevailing patterns of the response variables in relation to 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 avoid the time effect, caused by the 4.5 month duration of the experiment, date was used as a co-variable. 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. All possible relationships among the four data sets were investigated. 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, which is most appropriate to explain another set of variables.

RESULTS

An overview of all the performed RDAs is listed in Table 1. The first canonical axis as well as the first four canonical axes combined were statistically significant at the 5% level for all RDAs. Table 2 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 was best 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.

Table 1. Results of the redundancy analyses (RDA) between all combinations of the datasets.

	Axis 1	Axis 2	Axis 3	Axis 4
Natural food availability in relation to water quality				
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
Rohu diet in relation to natural food availability				
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

Table 1 (Continued)

	Axis 1	Axis 2	Axis 3	Axis 4
CC diet in relation to natural food availability				
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
Rohu growth in relation to rohu diet				
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	0.0
CC growth in relation to CC diet				
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	0.0
Rohu growth in relation to water quality				
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	0.0
CC growth in relation to water qualitty				
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	0.0

Total variance =1.000; all RDAs were statistically significant at $P<0.05$). CC = common carp

Table 2. Correlations among the datasets along the first RDA axis (r value).

	Response variables			
Explanatory 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.

Food availability in relation to water quality

The outcomes of the RDA are presented in Table 1 and Figure 1. In the RDA, two significant axes explained 49.7% of the variance in natural food availability and 91.7% of the natural food-water quality relation. The first RDA axis was positively correlated with the presence of CC (Figure 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}.

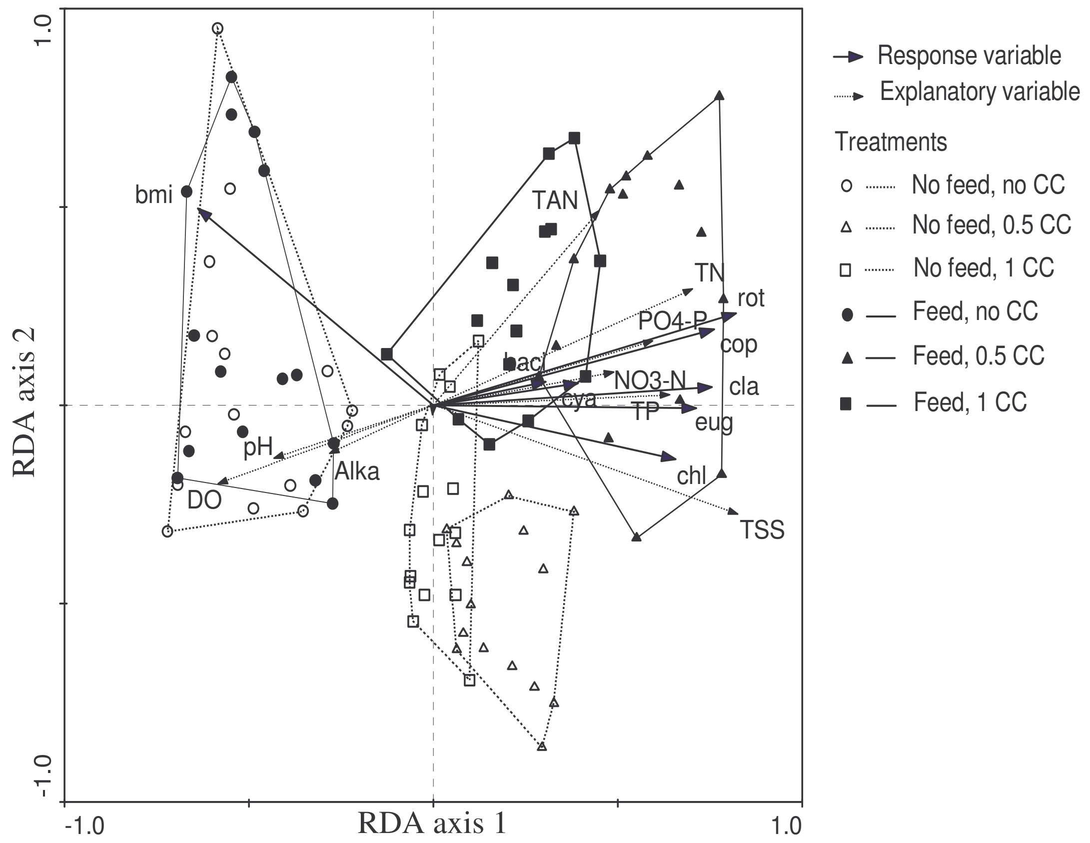

Figure 1. RDA biplot (first two axes) of natural food availability in the pond explained by pond water quality (baci = total Bacilariophyceae, chl = total Chlorophyceae, cya = total Cyanophyceae, eug = total Euglenophyceae, rot = total Rotifera, cla = total Cladocera, cop = total Copepoda, bmi = total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

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 90^0 indicates independence of variables, and an angle larger than 90^0 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 in the pond sediments. The density of benthic macroinvertebrates was positively correlated with total alkalinity, pH and DO concentration whereas negatively correlated with all other water quality parameters.

Diet in relation to 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 1). In rohu, the treatments with artificial feed addition scored high on the first RDA axis (Figure 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 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 (Figure 2). In the absence of artificial feed, the treatment with 0.5 CC m^{-2} scores 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 is higher, but this appears not to a clear relationship with the diet. Only the amount of ingested Bacillariophyceae was higher in case of the higher CC density.

Fish growth in relation to 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 (Table 1). In rohu, the treatments with artificial feed addition scored high on the first RDA axis (Figure 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 Euglenaphyceae) and growth variables. Rohu growth variables were strongly

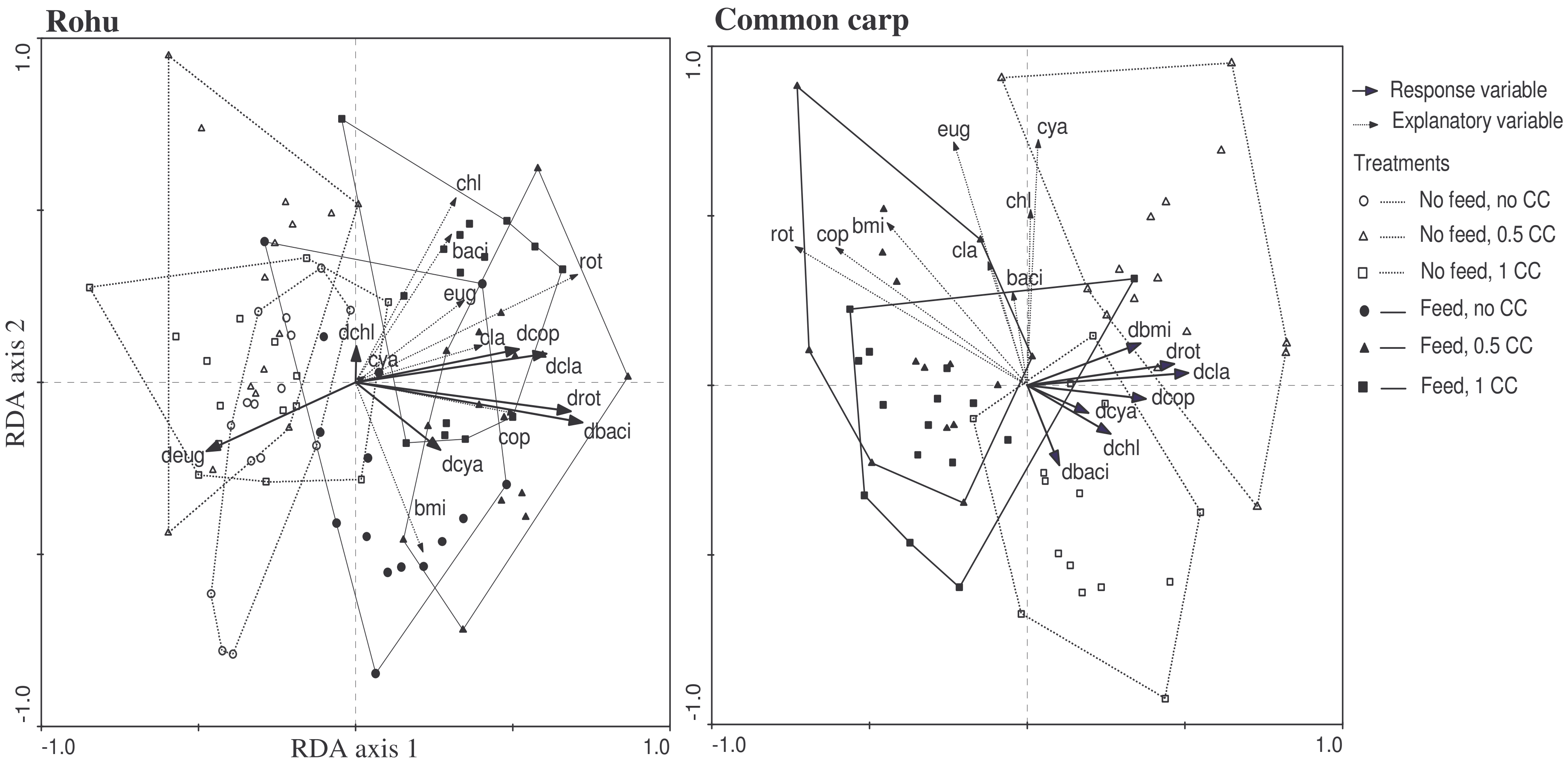

Figure 2. RDA biplot (first two axes) of fish diet explained by food availability in the ponds water (baci = total Bacilariophyceae, chl = total Chlorophyceae, cya = total Cyanophyceae, eug = total Euglenophyceae, rot = total Rotifera, cla = total Cladocera, cop = total Copepoda and bmi = total benthic 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).

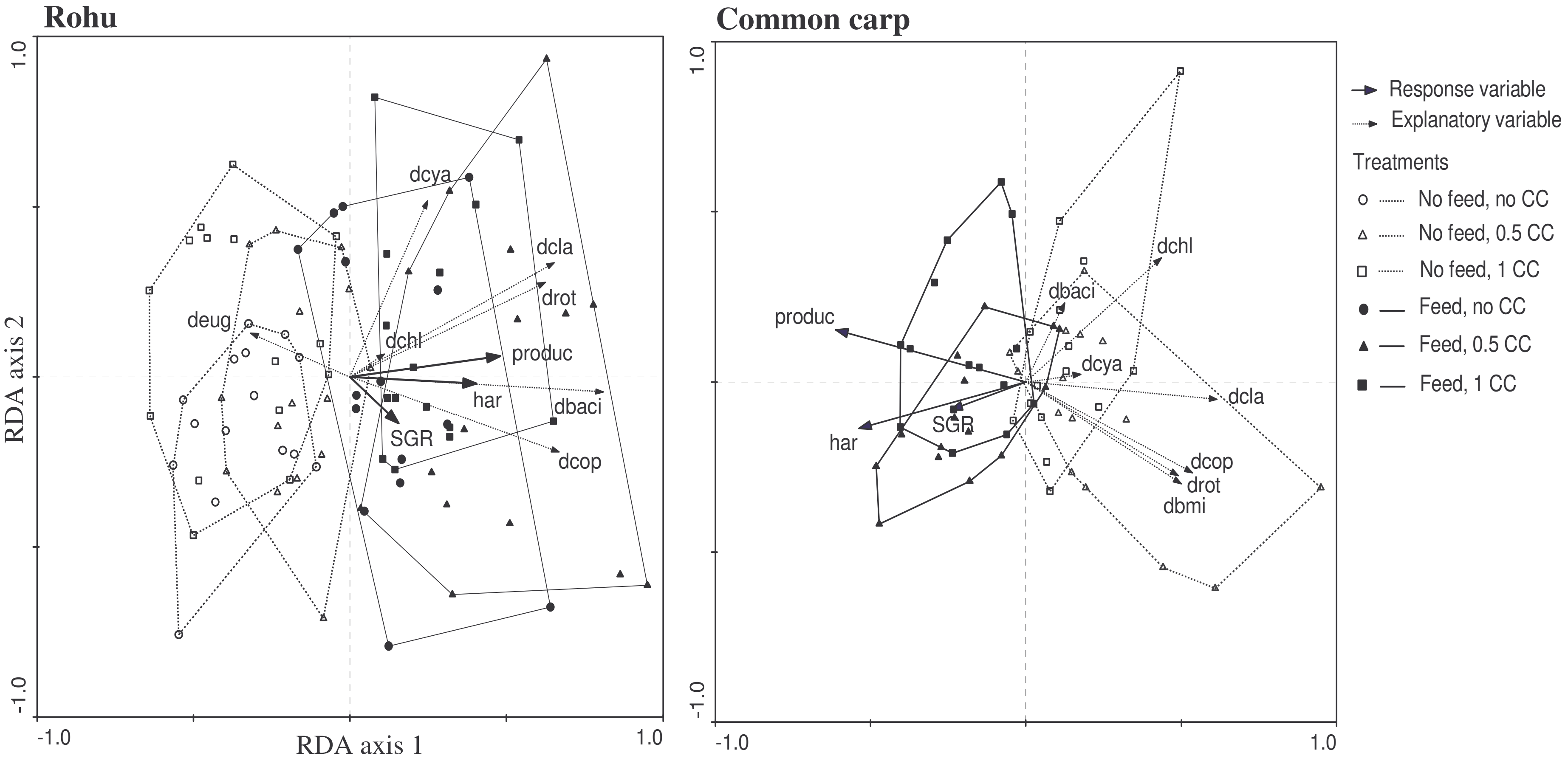

Figure 3. RDA biplot (first two axes) of growth explained by fish diet (dbaci = total Bacilariophyceae, dchl = total Chlorophyceae, dcya = total Cyanophyceae, deug = total Euglenophyceae, drot = total Rotifera, dcla = total Cladocera, dcop = total Copepoda, dbmi = total benthic macroinvertebrate, har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

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 (Figure 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 is higher than that of phytoplankton ingestion.

Fish growth in relation to 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 1). In rohu, the treatments with CC addition scored higher on the first RDA axis (Figure 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 results in higher RDA scores. This effect is 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 is 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 is stronger in the cases with 0.5 CC m^{-2} than in those with 1 CC m^{-2}. 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.

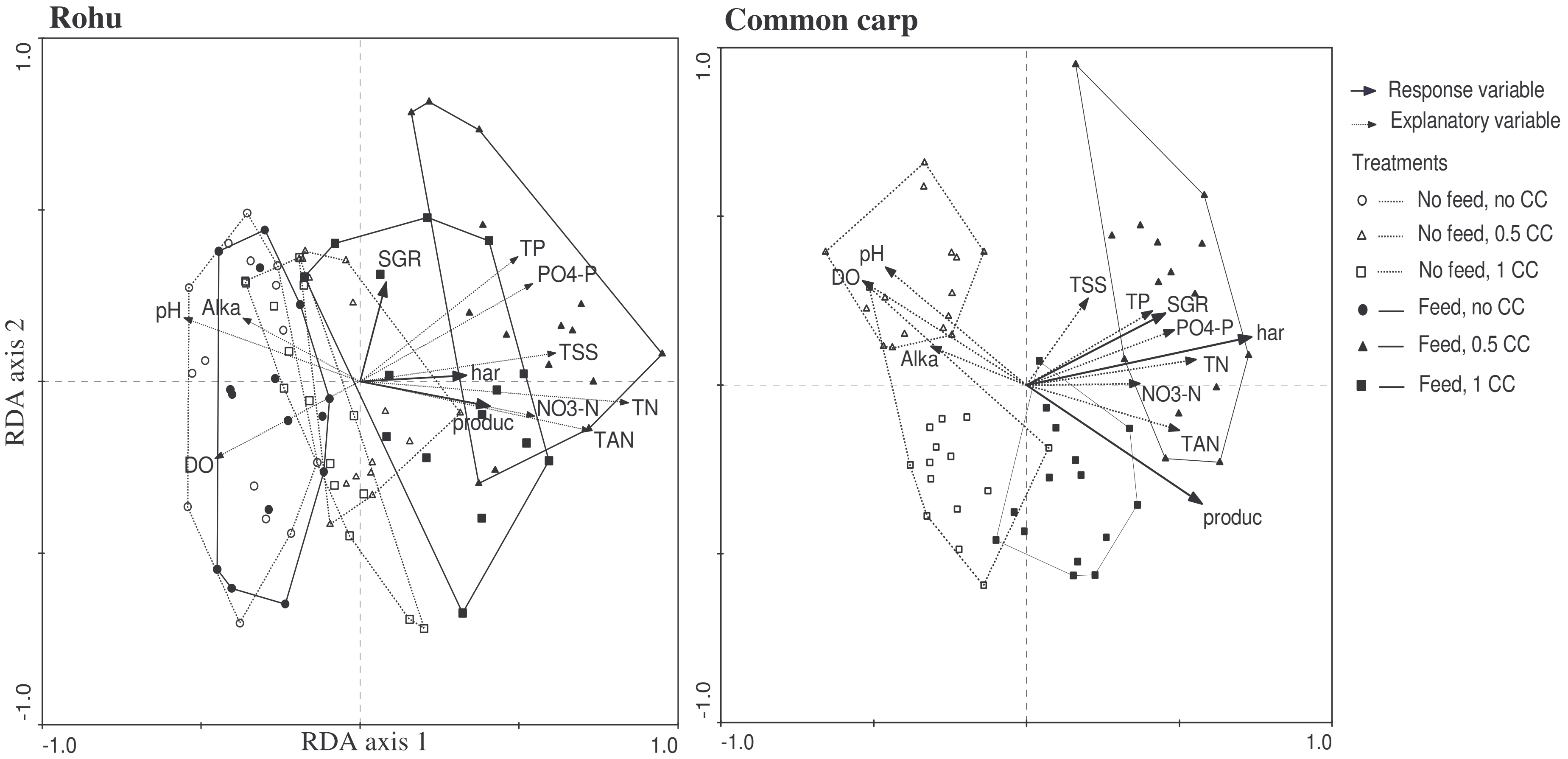

Figure 4. RDA biplot (first two axes) of fish growth explained by pond water quality (har = average harvesting weight of fish, produc = total production of fish and Alka = total alkalinity).

DISCUSSION

This study shows the overall patterns of the effects of feed addition and/or 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 data set 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 (Figure 1). These observations are in a way in concordance with Milstein et al. (2002), Parkos III et al. (2003) and Ritbo 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 and fish 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}. However, 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 (Figure 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 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 Figure 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 at el. (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 were most probably caused by, (i) the respiration of zooplankton, and (ii) decomposition of organic matter produced by the zooplankton. The similar negative correlation between zooplankton availability and DO concentration was also documented by Aka et al. (2000) and Dresilign (2003).

We found that for rohu the overall diet was better explained by food availability (correlation along the first RDA axis: r = 0.835) than by any of the other datasets (Table 2). Rohu increased natural food ingestion (except Euglenophyceae) with increasing natural food availability in the pond. However, both the availability and the ingestion were higher if artificial feed was added (Figure 2). Almost similar effects of artificial feed are also observed for the relationship between natural food ingestion and growth and production of rohu (Figure 3), which closely correlated with the ingestion of natural food (Table 2: correlation along the first RDA axis: r = 0.787). 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 (Diana et al., 1997; Dewan et al., 1988), increasing the amount of natural food through the higher concentrations of nutrients in presence of common carp (Figure 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 correlation between availability and ingestion of total zooplankton (r = 0.57, $P<0.01$) is higher than the correlation between the availability and ingestion of total phytoplankton (r = 0.41, $P<0.01$) in rohu. Moreover, the effect of the ingestion variables of all zooplankton groups on the overall ordination is higher than the effects of almost all phytoplankton ingestion variables (except for Bacillariophyceae). These results indicate that rohu reacts more strongly on changes in zooplankton availability, suggesting that rohu prefers zooplankton over phytoplankton. This result confirms our univariate analysis and in a way agrees with Miah et al. (1984), who mentioned rohu fry prefer zooplankton above phytoplankton.

In CC, we found that the overall diet was better explained by food availability (correlation along the first RDA axis: r = 0.766) than by any of the other datasets (Table 2), 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 relatively lower (r = 0.767 along the first RDA axis; Table 2), than the relationship between water quality and growth and production datasets (r = 0.927 along the first RDA axis; Table 2), 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 (Figure 2, 3). These results indicate that common carp prefers artificial feed over natural foods. In absence of artificial feed, CC preferred zooplankton and benthic macroinvertebrate 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} (Figure 2). This result in a way agrees with Spataru et al. (1983), Sibbing (1988) and Garcia-Berthou (2001), who mentioned CC is a bottom feeder feeding on zooplankton and benthic macroinvertebrate 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).

In this study we directly related four different datasets to analyse how variation in one dataset could be explained by variation in other datasets. We started from the hypothesis that water quality determines natural food availability, which in turn explains natural food ingestion, the latter explaining fish growth and production. However, also other interactions might play a role, for instance water quality might directly influence growth performance. Therefore, all combinations of datasets for both CC and rohu were studied (Table 2). For rohu, the strongest correlative links found in the dataset were exactly the steps from the hypothesis. This stepwise approach explained more of the total variance than separate correlations between data sets would have revealed. For CC, it was found that although natural food availability is still the best explanatory dataset for natural food ingestion, the latter is not the best explanatory dataset for growth and production of CC. Water quality explains the variation in growth and production of CC better in this study. This indicates that other mechanisms than the steps that we formulated are dominating the growth and production of CC. The fact that artificial feed was not used as a variable and was not scored as a food item, while it is the preferred food of CC explains the low explanatory power of natural food intake for growth and production. Results showed that the pair wise comparisons of several datasets are a promising tool for elucidating causal links in pond aquaculture research.

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Chapter 3

Growth, production and food preference of rohu *Labeo rohita* (H.) in monoculture and in polyculture with common carp *Cyprinus carpio* (L.) under fed and non-fed ponds

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ABSTRACT

An experiment was carried out in 18 earthen ponds to investigate the effects of the addition of common carp *Cyprinus carpio* (L.) and artificial feed on natural food availability, food utilization and fish production in rohu *Labeo rohita* (Hamilton) ponds. Ponds were fertilized fortnightly with cow manure, urea and triple super phosphate. Rohu was stocked in all ponds at a density of 1.5 rohu m^{-2}. All treatments were carried out in triplicate. Treatments were: rohu with and without formulated feed, rohu plus 0.5 common carp m^{-2} with and without feed, and rohu plus 1 common carp m^{-2} with and without feed. The time period between stocking and harvesting was four and half months. Stocking 0.5 common carp m^{-2} enhanced natural food availability in the pond, food utilization and rohu growth and production ($P<0.05$). The effect was less pronounced when stocking 1 common carp m^{-2}. Formulated feed administration did not influence phytoplankton availability ($P>0.05$) but increased zooplankton and benthic macroinvertebrate availability ($P<0.001$). Feed administration also enhanced growth of rohu and common carp ($P<0.001$). Rohu naturally ingests more phytoplankton than zooplankton but in the presence of formulated feed rohu shifted its natural food preference from phytoplankton to zooplankton. Common carp naturally ingests mainly zooplankton and benthic macroinvertebrate and small quantities of phytoplankton. However, when offered formulated feed, the latter becomes the preferred food item.

***Keywords**:* Food preference; Food availability; Polyculture; Pond; Rohu; Common carp.

INTRODUCTION

Semi-intensive carp polyculture is an age-old popular practice in south Asia, especially in Bangladesh and India, where it is the main aquaculture production system (Miah et al., 1997; FAO, 1997; Reddy et al., 2002). Between July 1999 and July 2000 the total inland fish production of Bangladesh was about 650,000 metric tons. More than 80% of this production was realized in semi-intensive culture systems (DOF, 2001). The key characteristic of these systems is the reliance on the combination of natural and artificial feed (Hepher and pruginin, 1982; Moore, 1985). In addition, polyculture is preferred, based on the assumption that each species stocked has its own feeding niche that does not completely overlap with the feeding niche of other species. In consequence, a larger fraction of the natural food available in the pond is used in multi-species systems. In some cases, one species enhances the food available for other species, thus increasing further the total fish yield per unit area (Swingle, 1966; Hepher et al., 1989; Miah et al., 1993).

In south Asian polyculture, a wide variety of fish species are cultured. Among those species, rohu *Labeo rohita* (Hamilton), catla *Catla catla (*Hamilton) and mrigal *Cirrhinus cirrhosus* (Bloch) are very popular (Uddin et al., 1994; FAO, 1997; Miah et al., 1997; Kanak et al., 1999). Between July 1998 and July 1999, these 3 species contributed 70% of the total inland aquaculture production in Bangladesh (DOF, 2001). Nevertheless, production systems are continuously changing. Nowadays, farmers prefer to stock rohu because rohu enjoys a higher consumer preference and market value. The farmers also prefer to stock common carp *Cyprinus carpio* (L.) as a bottom feeder instead of mrigal because common carp grows faster than mrigal and the overall production is higher when combined with rohu and catla in polyculture ponds (Dewan et al., 1985; Wahab et al., 1995; Milstein et al., 2002). Wahab et al. (2002) conducted an experiment with rohu, catla, punti *Puntius sophore* (Hamilton), common carp and mrigal in semi-intensive polyculture and achieved a 60% higher yield of rohu with common carp as bottom feeder compared to mrigal.

Rohu is known as a water column feeder mainly feeding on plankton (Das and Moitra, 1955; Dewan et al., 1977; Jhingran and Pullin, 1985; Wahab et al., 1994) and common carp is a bottom feeder mainly feeding on benthic macroinvertebrate and zooplankton (Tang, 1970; Spataru et al., 1980; Hepher and Pruginin, 1981; Spataru et al., 1983). When artificial feed is applied, common carp readily accepts artificial feeds (Yashov and Halevy, 1972; Spataru et al., 1980; Schroeder, 1983; Milstein and Hulata, 1993). The food and feeding habits of rohu and common carp might differ according to the overall food and feed availability. Stirring effect of common carp may enhance nutrient availability, which in turn increase natural food availability in the ponds (Yashouv, 1971; Milstein et al., 1988; Milstein et al., 2002). Such principles are commonly accepted but have never been well quantified. However, the quantitative effects of common carp density on food availability and feed intake remain unclear (Wahab et al., 1995; Milstein et al., 2002). Indeed, most studies, which tried to analyze the quantitative relation of food web ecology of polyculture ponds rely on percent occurrence numbers of different food items in the gut (Dewan et al., 1991; Wahab et al., 1995; Azim et al., 2004). However, due to the large size variation of the natural food item, these data provide a poor indication of the actual biomass present in the pond or ingested by fish. Volumetric or weight measurements of gut content is a better indicator of the contribution to total food intake. Considering the above issues, the goal of this study was to elucidate the effects of artificial feed and addition of common carp on natural food availability, food preference, food intake, growth and fish production in semi-intensive rohu ponds.

MATERIALS AND METHODS

Study site and pond preparation

The 4.5 months experiment was conducted between March and July 2003 in 18 earthen ponds located at the Fisheries Faculty Field Laboratory, Bangladesh Agricultural University, Mymensingh, Bangladesh. All ponds were rectangular in shape with a size of 100 m^2 and an average depth of 1.2 m. The ponds were supplied by groundwater from an adjacent deep tube-well. Prior to the experiment, ponds were drained, renovated, aquatic vegetation was removed and fishes and macrofauna were eradicated. All ponds were treated with agricultural lime ($CaCO_3$) at a rate of 250 kg ha^{-1} and filled with water 7 days prior to fertilization. 1,250 kg ha^{-1} of decomposed cow manure, 31 kg ha^{-1} of urea and 16 kg ha^{-1} of triple super phosphate (TSP) were applied to all ponds one week before stocking, followed by fortnightly applications of the same amounts for the duration of the study period.

Experimental design

A factorial design was used, the factors being common carp stocking density (3 levels: 0, 0.5 and 1 common carp m^{-2}) and feed addition (2 levels: feed addition and no feed addition). All ponds were stocked with 1.5 rohu m^{-2}. The combinations of the two factors resulted in the following 6 treatments: rohu alone without (formulated) feed (treatment 0C), rohu alone with feed (0C-F), rohu plus 0.5 common carp m^{-2} without feed (0.5C), rohu plus 0.5 common carp m^{-2} with feed (0.5C-F), rohu plus 1 common carp m^{-2} without feed (1C), and rohu plus 1 common carp m^{-2} with feed (1C-F). All treatments were executed in triplicate.

Fish stocking and management

Rohu (mean individual stocking weight per pond ranged between 20.3-21.1 g) and common carp (20.6-21.4 g) fingerlings collected from a nearby nursery were released into the ponds in the afternoon. The 30% protein diet contained fish meal (protein: 57.5%, inclusion in feed 37%) rice bran (14%, 47%), mustard oil cake (14%, 15%) and vitamin premix (0%, 1%), and was applied daily at 15 g $kg^{-0.8}$ day^{-1} starting the day after stocking until the day prior to harvesting. Feeding rates per pond were adjusted monthly after weighing minimum 20% of the fishes stocked.

Assessment of plankton and benthic macroinvertebrate

Water samples for plankton analysis were collected fortnightly taking 1 L samples at 10 different locations in each pond with a Niskin sampler. Each composite 10 L sample was then passed through a 10-µm mesh plankton net. Each concentrated plankton sample was then transferred to a plastic bottle and diluted to 100 ml with formalin and distilled water to obtain a 5% buffered formalin solution. Plankton numbers were estimated in a Sedgewick-Rafter (S-R) cell. A 1 ml sample was put in the S-R cell and was left 10 minutes 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. As determination keys were used 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 planktonic organisms per liter of pond water; P = the number of planktonic organisms counted in ten fields; C = the volume of the plastic bottle holding the sample (100 ml); L = the volume of the pond water sample (10 L). The benthic macroinvertebrate samples were collected fortnightly with an Ekman dredge. In each pond, bottom mud samples were collected from 3 different locations and washed through a 250 µm mesh size sieve. Benthic macroinvertebrate remaining on the sieve was preserved in a plastic vial containing a 10% buffered formalin solution. Identification keys used for benthic macroinvertebrate were Brinkhurst (1971) and Pinder and Reiss (1983). Benthic macroinvertebrate density was calculated using the formula,

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

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

Analysis of gut content

One fish per species per pond was collected each month for gut content analysis. Each fish was weighed individually and killed in ice water. The body cavity of the fish was carefully opened and 5 cm of the anterior gut was cut and preserved immediately in a jar containing 10% buffered formalin. The content from each 5 cm gut was carefully washed into a Petri dish and diluted to 50 ml with water. A 1 ml sub-sample was transferred by a pipette to a S-R cell containing 1000 1-mm^3 fields and left for 10 minutes to allow the solid particles to settle. The S-R cell was set up under a microscope and identification of food items was done in 10 square fields. The different food items collected from the gut were qualified and quantified up to the genus level. The calculations were done using the following formula,

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

with N = no. of a specific food item available in 5 cm gut, P = total no. of specific food observed in 10 fields, and C = volume (ml) of sample in the Petri dish.

Fish harvesting

At the end of the experiment ponds were drained and all fish were harvested and weighed up to the nearest 0.1 g. Specific growth rate (% body weight day^{-1}) was calculated using the formula,

$$SGR = [\ln WT_F - \ln WT_I] \times 100/T$$ (Hopkins, 1992)

with WT_F = average final fish weight (g), WT_I = average initial fish weight (g), T = duration of the experiment (days).

Data calculations and analysis

The biovolumes of plankton and benthic macroinvertebrate were calculated using literature values (see Table 1). Zooplankton biovolumes were calculated using length in their respective length/volume formula. In some cases, biovolume approximations were made using the values of species of similar shape.

Percent data were arcsine transformed before analysis, but non-transformed data are shown in Tables or Figures. All data were analyzed through two-way split-plot ANOVA except growth and yield data of fish. The two-way split-plot ANOVA was performed with common carp density and artificial feed as main factors and time as sub-factor (Gomez and Gomez, 1984). Fish growth and yield data were analyzed by two-way ANOVA performing with the main factors (common carp density and artificial feed). Because of the scope of the present paper, we will discuss only the effects of main factors. The sub-factor time and its interactions with the main factors common carp densities and artificial feed addition, will be discussed in a separate article.

Table 1. Phytoplankton and zooplankton species individual biovolume as used to convert numbers into total biovolume.

Group	Genus	Organism volume (μm^3)	References	Assumption
Bacillariophyceae	*Achanthes*	600	-	*Cyclotella*
	Actinella	600	-	*Cyclotella*
	Cocconeis	600	-	*Cyclotella*
	Cymbella	600	-	*Cyclotella*
	Cyclotella	600	Berman and Pollingher, 1974.	-
	Fragilaria	810	Peerapornpisal, 1996.	-
	Frustrularia	810	-	*Fragilaria*
	Gomphonema	600	-	*Cyclotella*
	Melosira	910	Berman and Pollingher, 1974.	-
	Navicula	850	Berman and Pollingher, 1974.	-
	Nitzschia	1,240	Peerapornpisal, 1996.	-
	Surirela	2,630	-	*Phacus*
	Synedra	1,240	-	*Nitzschia*
	Tabellaria	1,240	-	-
Chlorophyceae	*Ankistrodesmus*	52	Beveridge et al., 1993.	-
	Actinustrum	52	-	*Ankistrodesmus*
	Bothriococcus	6,190	Peerapornpisal, 1996.	-
	Chaetophora	1,326	-	*Anabaena*
	Chlorella	30	Berman and Pollingher, 1974.	-
	Chrysococcus	6,190	-	*Bothriococcus*
	Closteridium	335	-	*Closterium*
	Closterium	1,675	Peerapornpisal, 1996.	-
	Coelastrum	1,208	Peerapornpisal, 1996.	-
	Cosinodiscus	1,208	-	*Coelastrum*
	Crucigenia	220	Peerapornpisal, 1996.	-
	Elakatothrix	15	Peerapornpisal, 1996.	-
	Geminella	1,326	-	*Anabaena*
	Gonatozygon	1,326	-	*Anabaena*
	Haematococcus	6,190	-	*Bothriococcus*
	Merismopedia	896	-	*Pediastrum*
	Microspora	896	-	*Pediastrum*
	Oedogonium	896	-	*Pediastrum*
	Oocystis	250	Berman and Pollingher, 1974.	-
	Pediastrum	896	Peerapornpisal, 1996.	-
	Pleurococcus	6,190	-	*Bothriococcus*
	Scenedesmus	115	Berman and Pollingher, 1974.	-
	Selenastrum	115	-	*Scenedesmus*
	Sprogyra	1,326	-	*Anabaena*
	Sphaerocystis	896	-	*Pediastrum*
	Tetraedron	85	Berman and Pollingher, 1974.	-
	Tetraspora	90	-	*Tetraedron*

Table 1 (continued)

Group	Genus	Organism volume (μm^3)	References	Assumption
Chlorophyceae	*Ulothrix*	1,326	-	*Anabaena*
	Volvox	30	-	*Chlorella*
	Zignema	1,326	-	*Anabaena*
Cyanophyceae	*Anacystis*	1,326	-	*Anabaena*
	Anabaena	1,326	Beveridge et al., 1993.	-
	Aphanizomenon	1,231	Peerapornpisal, 1996.	-
	Aphanocapsa	1,231	-	*Aphanizomenon*
	Aphanotheca	1,231	-	*Aphanizomenon*
	Chroococcus	280	Berman and Pollingher, 1974.	-
	Gomphospheria	1,208	-	*Coelastrum*
	Coelospharium	1,208	-	*Coelastrum*
	Gleocapsa	1,208	-	*Coelastrum*
	Microcystis	11,300	Peerapornpisal, 1996.	-
	Lyngbya	1,326	-	*Anabaena*
	Oscillatoria	1,326	-	*Anabaena*
Euglenophyceae	*Euglena*	1,956	Peerapornpisal, 1996.	-
	Phacus	2,630	Peerapornpisal, 1996.	-
	Trachelomonas	5,089	Peerapornpisal, 1996.	-
Rotifera	*Asplanchna*	483,840	-	*Polyarthra*
	Brachionus	483,840	-	*Polyarthra*
	Filinia	483,840	-	*Polyarthra*
	Polyarthra	483,840	McCauley, 1984; Bottrell et al., 1976.	Length (L) = 120 μm volume = 0.28 L^3
	Keratella	944,023	Bottrell et al., 1976; McCauley, 1984.	Length (L) = 162.5 μm, Volume = 0.22 L^3
	Trichocerca	483,840	-	*Polyarthra*
Cladocera	*Daphnia*	483,840	-	*Polyarthra*
	Diaphanosoma	483,840	-	*Polyarthra*
	Moina	483,840	-	*Polyarthra*
Copepoda	*Diaptomus*	483,840	-	*Polyarthra*
	Nauplius	944,023	-	*Keratella*
	Monostyla	483,840	-	*Polyarthra*
	Cyclops	944,023	-	*Keratella*
Oligocheta	-	1.273 (mm^3)	Riera et al., 1991.	Volume = $\pi LD^2/4$, with D = average diameter (0.4 mm), L = length (10.13 mm), L = -1.408 + 28.835D.
Chironomidae	-	1.273 (mm^3	-	Oligocheta

Genus name in the assumption column indicates assuming similar biovolume of the genus given in the same row. All assumptions were made on the basis of average size under microscopic observation.

RESULTS

Effects on plankton and benthic macroinvertebrate biovolume

Phytoplankton samples contained four major groups (Bacillariophyceae, 14 genera; Chlorophyceae, 31; Cyanophyceae, 13 and Euglenophyceae, 3), zooplankton three (Rotifera, 6; Cladocera, 3 and Copepoda, 4), and benthic macroinvertebrate two (Oligochaeta and Chironomidae) (Table 2). In all treatments the same genera of plankton were found. The results of the ANOVA on the biovolume of major groups of plankton and total benthic macroinvertebrate are shown in Table 3. Common carp density affected the biovolume of all the groups of phytoplankton, zooplankton and benthic macroinvertebrate except Bacillariophyceae. The mean total biomass of Chlorophyceae, Cyanophyceae, Euglenophyceae and total phytoplankton were higher in ponds with 0.5 than in ponds with 1 and 0 common carp per m^{-2}. In the case of Rotifera, Cladocera, Copepoda and total zooplankton, the mean total biomass was the highest with 0.5 common carp m^{-2}, followed by 1 and 0 common carp m^{-2}. In contrast, when considering the effects of common carp on benthic macroinvertebrate biomass, higher biomass was observed in treatments without common carp, followed by treatments with 0.5 and 1 common carp m^{-2}. The benthic macroinvertebrate biomass in the treatment without common carp was 1.7 and 1.9 times higher than in treatments with 0.5 and 1 common carp m^{-2}, respectively.

The effect of artificial feed addition was not significant on any phytoplanktonic group but was significant for all zooplanktonic groups and total benthic macroinvertebrate biomass. The total zooplankton and benthic macroinvertebrate biomass in treatments with feed were 1.3 times higher than in treatments without feed.

Table 2. List of plankton and macroinvertebrate recorded from the experimental pond water and gut content of rohu and common carp.

Group	Genus	Pond water	Rohu gut	Common carp gut
Bacillriophyceae				
	Achanthes	×		
	Actinella	×		
	Cocconeis	×	×	×
	Cyclotella	×	×	×
	Cymbella	×	×	×
	Fragilaria	×	×	×
	Frustularia	×		
	Gomphonema	×	×	×
	Melosira	×	×	×
	Navicula	×	×	×
	Nitzschia	×	×	×
	Surirela	×	×	×
	Synedra	×	×	×
	Tabellaria	×	×	
Chlorophyceae				
	Actinastrum	×	×	
	Ankistrodesmus	×	×	×
	Botryococcus	×	×	×
	Chaetophora	×	×	×
	Chlorella	×	×	×
	Chrysococcus	×	×	×
	Closteridium	×	×	×
	Closterium	×	×	×
	Coelastrum	×	×	×
	Coscinodiscus	×	×	×
	Crucigenia	×	×	×
	Elakatothrix	×		
	Geminella	×	×	×
	Gonatozygon	×	×	×
	Haematococcus	×		
	Merismopedia	×	×	×
	Microspora	×	×	
	Oedogonium	×	×	×
	Oocystis	×	×	×
	Pediastrum	×	×	×
	Pleurococcus	×	×	×
	Pleurosigma	×	×	×
	Scenedesmus	×	×	×
	Selenastrum	×	×	
	Sphaerocystis	×		
	Sprogyra	×	×	×

Table 2 (continued)

Group	Genus	Pond water	Rohu gut	Common carp gut
Chlorophyceae	*Tetraedron*	×	×	×
	Tetraspora	×	×	
	Ulothrix	×	×	×
	Volvox	×	×	×
	Zignema	×	×	×
Cyanophyceae				
	Anabaena	×	×	
	Anacystis	×	×	×
	Aphanizomenon	×	×	×
	Aphanocapsa	×	×	×
	Aphanothece	×	×	×
	Chroococcus	×	×	×
	Coelospharium	×		×
	Gleocapsa	×		
	Gomphospheria	×	×	×
	Lyngbya	×	×	×
	Microcystis	×	×	×
	Oscillatoria	×	×	×
Euglenophyceae				
	Euglena	×	×	
	Tracchelomonas	×		
	Phacus	×	×	
Rotifera				
	Asplanchna	×	×	×
	Brachionus	×	×	×
	Filinia	×	×	×
	Keratella	×	×	×
	Polyarthra	×	×	×
	Trichocerca	×	×	×
Cladocera				
	Daphnia	×	×	×
	Diaphanosoma	×	×	×
	Moina	×	×	×
Copepoda				
	Cyclops	×	×	×
	Diaptomus	×	×	×
	Monostyla	×	×	×
	Nauplius	×	×	×
Macroinvertebrate				
Oligocheta		×		×
Chironomideae		×		×

“×” indicates presence.

Table 3. Effects of common carp and supplementary feed on the abundance (based on total volume, mm^3 L^{-1}) of different groups of plankton and macroinvertebrate in ponds based on two-way ANOVA.

Variable	Significance (P value)			Tukey test				
				Common carp density			Feed	
	CC	Feed	CC×Feed	0	0.5	1	Yes	No
Bacillariophyceae	NS	NS	NS	0.039	0.041	0.041	0.041	0.040
Chlorophyceae	***	NS	NS	0.122^{b}	0.147^{a}	0.131^{b}	0.135	0.131
Cyanophyceae	**	NS	NS	0.077^{b}	0.103^{a}	0.081^{b}	0.088	0.085
Euglenophyceae	***	NS	*	0.027^{b}	0.061^{a}	0.035^{b}	0.044	0.038
Total Phytoplankton	***	NS	NS	0.265^{b}	0.352^{a}	0.287^{b}	0.308	0.294
Rotifera	***	***	***	0.026^{c}	0.038^{a}	0.035^{b}	0.037^{a}	0.029^{b}
Cladocera	***	**	NS	0.007^{c}	0.014^{a}	0.010^{b}	0.011^{a}	0.009^{b}
Copepoda	***	**	*	0.009^{c}	0.016^{a}	0.013^{b}	0.014^{a}	0.011^{b}
Total Zooplankton	***	***	**	0.042^{c}	0.067^{a}	0.057^{b}	0.062^{a}	0.049^{b}
Total Macroinvertebrate in bottom (cm^3 m^{-2})	***	***	**	6.242^{a}	3.730^{b}	3.221^{c}	4.954^{a}	3.840^{b}

CC = common carp density; Feed = feed addition; CC×Feed = interaction of common carp density and feed. Mean values in the same row with no superscript in common differ significantly ($P<0.05$). If the effects are significant, ANOVA was followed by Tukey test. *$P\leq0.05$; **$P<0.01$; ***$P<0.001$; NS, not significant.

Effects on gut content

The gut content of rohu and common carp consisted of phytoplankton (rohu 51 genera and common carp 46), zooplankton (both species 13), benthic macroinvertebrate (rohu 0 and common carp 2 groups) (Table 2) and unidentified particles. Unidentified particles were more abundant in the treatments with feed than in the treatments without feed. Benthic macroinvertebrate was found in the gut of common carp but not in rohu. Euglenophyceae were only found in the gut of rohu. The results of two-way ANOVA on the biovolume of the major groups of plankton in the guts of rohu and common carp are shown in Tables 4 and 5, respectively. The biovolume of total phytoplankton and total zooplankton in the gut of rohu was higher with 0.5 than with 0 or 1 common carp m^{-2}. There was no effect of common carp density on the biovolume of all phytoplanktonic groups ($P>0.05$) in the gut of common carp. The biovolume of total zooplankton and benthic macroinvertebrate in the gut of common carp was significantly ($P<0.05$) higher in ponds with 0.5 than in ponds with 1 common carp m^{-2}.

Feed addition had significant effects on rohu’s ingestion of all groups of phytoplankton and zooplankton except Chlorophyceae. The ingestion of Bacillariophyceae, Cyanophyceae, Rotifera, Cladocera and Copepoda was higher in treatments with feed than in the treatments without feed, whereas an opposite result was observed in case of the ingestion of Euglenophyceae. The volume of total phytoplankton ingested by rohu in treatments with feed was 1.3 times higher than in treatments without feed. Again, the quantity of zooplankton ingested by rohu in treatments with feed was 3.2 times higher than in treatments without feed. In case of common carp, the artificial feed had significant effects on the ingestion of all planktonic groups and benthic macroinvertebrate except Bacillariophyceae, but this effect was opposite to the effect in rohu. The ingestion of total phytoplankton and total zooplankton by common carp in treatments without feed was 1.3 and 1.7 times higher, respectively, than in treatments with feed. In general, common carp ate more benthic macroinvertebrate than plankton. For benthic macroinvertebrate too, intake was 1.9 times higher in treatments without feed than in treatments with feed.

Table 4. Effects of common carp and supplementary feed on the composition of gut content of rohu (based on total volume, mm^3) of different groups of plankton and macroinvertebrate based on two-way ANOVA.

Variable	Significance (*P* value)			Tukey test				
				Common carp density			Feed	
	CC	Feed	CC× Feed	0	0.5	1	Yes	No
Bacillariophyceae	**	***	NS	0.170^{ab}	0.199^{a}	0.147^{b}	0.263^{a}	0.081^{b}
Chlorophyceae	*	NS	NS	0.291^{b}	0.345^{a}	0.322^{a}	0.328	0.310
Cyanophyceae	NS	***	**	0.044	0.048	0.055	0.058^{a}	0.039^{b}
Euglenophyceae	NS	***	NS	0.078	0.082	0.062	0.053^{b}	0.095^{a}
Total Phytoplankton	**	***	NS	0.583^{b}	0.674^{a}	0.586^{b}	0.685^{a}	0.544^{b}
Rotifera	NS	***	NS	0.243	0.272	0.251	0.381^{a}	0.130^{b}
Cladocera	**	***	**	0.173^{b}	0.229^{a}	0.248^{a}	0.324^{a}	0.110^{b}
Copepoda	**	***	**	0.090^{c}	0.171^{a}	0.132^{b}	0.216^{a}	0.046^{b}
Total Zooplankton	**	***	*	0.506^{b}	0.672^{a}	0.632^{b}	0.920^{a}	0.286^{b}

CC = common carp density; Feed = artificial feed addition; CC×Feed = interaction of common carp density and feed. Mean values in the same row with no superscript in common differ significantly ($P<0.05$). If the effects are significant, ANOVA followed by Tukey test. *$P\leq0.05$; **$P<0.01$; ***$P<0.0001$; NS, not significant.

Table 5. Effects of common carp density and supplementary feed on the composition of gut content of common carp (based on total volume, mm^3) of different groups of plankton and macroinvertebrate based on two-way ANOVA.

Variable	Significance (P value)			Tukey test			
				Common carp density		Feed	
	CC	Feed	CC×Feed	0.5	1	Yes	No
Bacillariophyceae	NS	NS	NS	0.141	0.156	0.142	0.156
Chlorophyceae	NS	*	NS	0.178	0.204	0.157^b	0.225^a
Cyanophyceae	NS	**	*	0.058	0.055	0.051^b	0.062^a
Total Phytoplankton	NS	**	NS	0.377	0.415	0.350^b	0.443^a
Rotifera	*	**	NS	0.206^a	0.168^b	0.152^b	0.223^a
Cladocera	NS	**	NS	0.261	0.227	0.179^b	0.310^a
Copepoda	*	***	NS	0.285^a	0.234^b	0.176^b	0.344^a
Total Zooplankton	**	**	NS	0.753^a	0.629^b	0.506^b	0.876^a
Total Macroinvertebrate	*	**	NS	3.733^a	2.846^b	2.310^b	4.270^a

CC = common carp density; Feed = artificial feed addition; CC×Feed = interaction of common carp density and feed. Mean values in the same row with no superscript in common differ significantly ($P<0.05$). If the effects are significant, ANOVA followed by Tukey test. *$P\leq0.05$; **$P<0.01$; ***$P<0.0001$; NS, not significant.

Effects on yield parameters of rohu and common carp

Yield parameters of rohu and common carp are shown in Table 6. For rohu there were significant common carp stocking density and artificial feed main effects but no interaction effects on its average individual harvesting weight, survival, specific growth rate (SGR) and yield. For common carp, the main effects and their interaction were significant ($P<0.05$) for all growth parameters. Rohu performed better in the presence of 0.5 common carp m^{-2} compared to the other common carp stocking densities (Table 6). Stocking 0.5 common carp m^{-2} resulted in a 1.4 times higher total rohu yield than stocking 0 or 1 common carp m^{-2}. Individual average harvesting weight was 1.5 times higher in treatments with 0.5 common carp m^{-2} than in the treatments with 1 common carp m^{-2}, but total common carp yield was 1.2 times higher in treatments with 1 common carp m^{-2} than the treatments with 0.5 common carp m^{-2}.

Yield of rohu and common carp was better in treatments with feed than in treatments without feed (Table 6 and Figure 1-2). For rohu, the total yield in treatments with feed was 1.5 times higher than in treatments without feed. For common carp, the total yield in treatments with feed was 2.1 times higher than in treatments without feed. The overall performance of rohu was better (highest average individual harvesting weight, 191.1 g; highest survival, 100%; highest specific growth rate, 1.62 % body wt. day^{-1}; highest total fish yield, 2860 kg ha^{-1} 137 day^{-1}) in treatment 0.5C-F, followed by treatment 1C-F, 0.5C, 0C-F, 0C and 1C. Total yield of rohu in treatment 0.5C-F was 1.3 times higher than in treatment 1C-F, 1.4 times higher than in treatment 0.5C and 0C-F, 2 times higher than in treatment 0C and 2.2 times higher than in treatment 1C. The overall performance of common carp was also better (highest average individual harvesting weight, 314.3 g; highest survival, 100% and highest specific growth rate, 1.97% body wt. day^{-1}) in treatment 0.5C-F, followed by treatment 1C-F, 1C and 0.5C. Total yield of common carp in treatment 1C-F was similar to treatment 0.5C-F but 1.8 times higher than in treatment 1C and 3 times higher than in treatment 0.5C.

Table 6. Effects of common carp density and supplementary feed on rohu and common carp average individual harvesting weight, survival, specific growth rate and yield, and total yield of fish (rohu plus common carp) in different treatments based on two-way ANOVA.

Variable	Significance (*P* value)			Tukey test				
				Common carp density			Feed	
	CC	Feed	CC×Feed	0	0.5	1.0	Yes	No
<u>*Rohu*</u>								
Average individual harvesting weight (g)	***	***	NS	139.1^{b}	173.4^{a}	132.9^{b}	164.9^{a}	132.0^{b}
Survival (%)	***	***	NS	83.5^{b}	94.5^{a}	84.7^{b}	94.4^{a}	80.7^{b}
SGR (% body weight days^{-1})	***	***	NS	1.39^{b}	1.55^{a}	1.35^{b}	1.51^{a}	1.35^{b}
Rohu yield (Kg ha^{-1} 137 days^{-1})	***	***	NS	1747^{b}	2473^{a}	1716^{b}	2343^{a}	1613^{b}
<u>*Common carp*</u>								
Average individual harvesting weight (g)	***	***	***	-	212.70^{a}	142.82^{b}	243.67^{a}	111.85^{b}
Survival (%)	***	***	**	-	99.3^{a}	90.7^{b}	98.7^{a}	91.3^{b}
SGR (% body weight day^{-1})	*	***	*	-	1.59^{a}	1.38^{b}	1.75^{a}	1.22^{b}
Common carp yield (Kg ha^{-1} 137 days^{-1})	*	***	*	-	1059^{b}	1314^{a}	1628^{a}	746^{b}
TOTAL YIELD (Kg ha^{-1} 137 days^{-1})	***	***	***	1747^{c}	3532^{a}	3030^{b}	3428^{a}	2111^{b}

CC = common carp density; Feed = artificial feed addition; CC×Feed = interaction of common carp density and feed. Mean values in same the row with no superscript in common differ significantly ($P<0.05$). If the effects are significant, ANOVA followed by Tukey test. * $P\leq 0.05$; ** $P<0.001$; ***$P<0.0001$; NS, not significant.

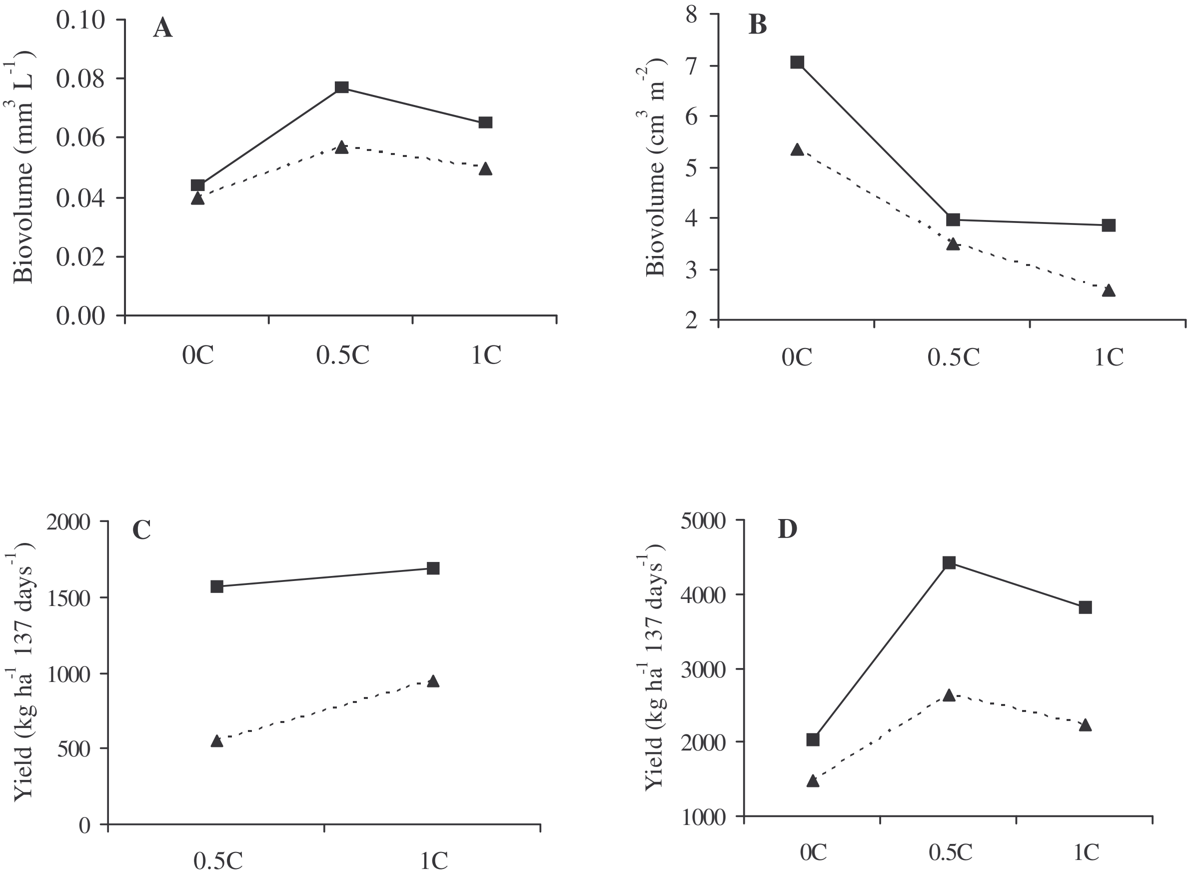

Figure 1. Interaction effects of common carp density and artificial feed on the abundance of total zooplankton in the water (A), on the abundance of total benthic macroinvertebrate in the bottom mud (B), total common carp yield (C) and total fish yield (D). 0C = treatments without common carp, 0.5C = treatments with 0.5 common carp m^{-2} and 1C = treatments with 1 common carp m^{-2}. Dotted and solid lines indicate treatments without and with feed, respectively.

Effects on combined fish yield

There were significant effects of common carp density, addition of feed and their interaction on the combined fish yield (Table 6). The combined fish yield in treatments with 0.5 common carp m^{-2} was 1.2 times higher than in treatments with 1 common carp m^{-2} and around 2 times higher than without common carp. The combined fish yield in treatments with feed was 1.6

times higher than in treatments with no feed. The combined yield of rohu and common carp and their contribution to this total combined yield are shown in Figure 2.

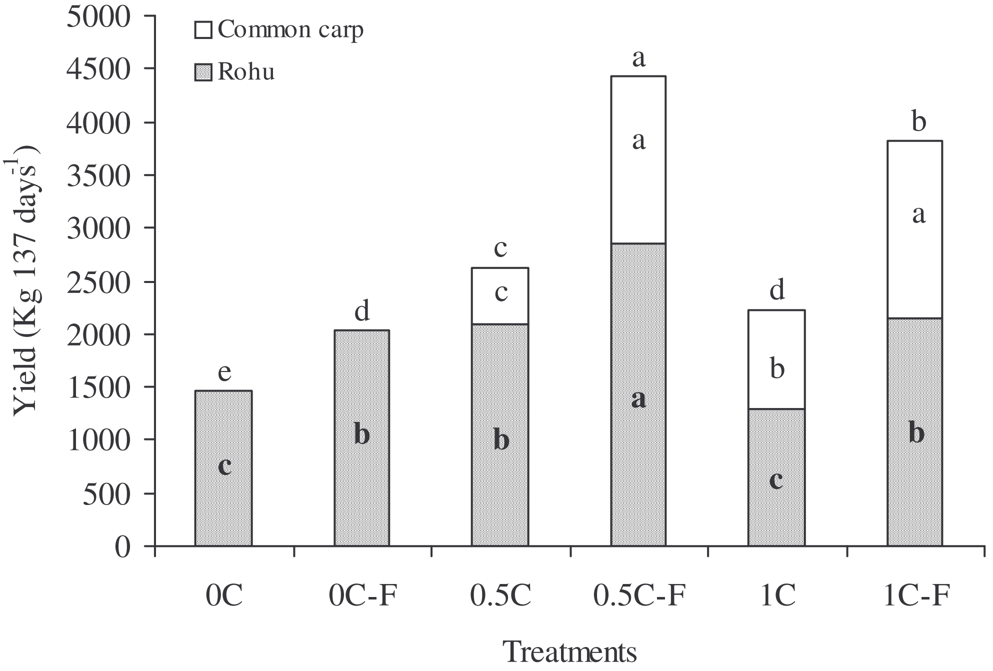

Figure 2. Total yield of fish and relative contributions of rohu and common carp in the six treatments. Letters above and within the bar relate to total yield and species yield, respectively based on Tukey test, one-way ANOVA. Yields with no latter in common are significantly different (*P*<0.05).

DISCUSSION

Effect on plankton and benthic macroinvertebrate biomass

The interaction between fish and food organisms is of utmost importance in polyculture systems (Milstein et al., 1988). Species with different feeding niches stocked at different densities can influence the natural food availability positively (e.g., by releasing nutrients from the pond bottom) or negatively (e.g., by direct ingestion) (Milstein, 1992; Milstein and Svirsky, 1996). According to Hepher et al. (1989) and Milstein and Svirsky (1996), stirring of sediments by common carp increases natural food availability by enhancing nutrient flows through the food web. A conceptual model of fish and food organism interactions, as influenced by common carp density and artificial feed using data from Table 3-6 is given in Figure 3. Browsing and burrowing for food by common carp helped to release nutrients from the bottom into the water column (Milstein et al., 1988; Milstein et al., 2002). The nutrients released stimulated photosynthesis, increasing phytoplankton and zooplankton biomass (Milstein, 1992; Wahab et al., 1995; Wahab et al., 2002). In this study, the effect of common carp addition on phytoplankton and zooplankton biomass was more pronounced with 0.5 common carp m^{-2} than with 1 common carp m^{-2} (central *vs* right sections of Figure 3). The possible reasons are: (1) more common carps increase turbidity (Meijer et al., 1990; Roberts et al., 1995; Parkos III et al., 2003), reducing photosynthesis and hence primary production (Hosseini and Oerdoeg, 1988); (2) higher grazing pressure by fish and zooplankton. Increasing common carp density may lead to overgrazing on natural foods, eventually up to the point that recovery is not possible (Steffens, 1990). Most likely in this experiment the grazing pressure and phytoplankton production were similar resulting in no further effects of common carp on phytoplankton biomass in treatments with 1 common carp m^{-2}.

The lower rate of primary production also affected zooplankton production, resulting in less zooplankton biomass in treatments with 1 compared to 0.5 common carp m^{-2} treatments. Less turbidity and grazing pressure improved primary and secondary production in treatments with 0.5 common carp m^{-2}. A different trend was observed in the benthic macroinvertebrate biomass, which decreased with increasing carp density as feeding pressure of common carp on benthic macroinvertebrate increased (Figure 3, comparison from left to right sections). This is in agreement with the results of Zur (1979), Riera et al. (1991), Tatrai et al. (1994), Zambrano and Hinojosa (1999).

In semi-intensive systems, artificial feed benefits the pond in two ways: it is either directly eaten by cultured animal or it indirectly supplies nutrients through decomposition by bacteria, fungi and protozoa (Moriarty, 1986; Milstein, 1992; Moriarty, 1997). In pond culture, 21% of

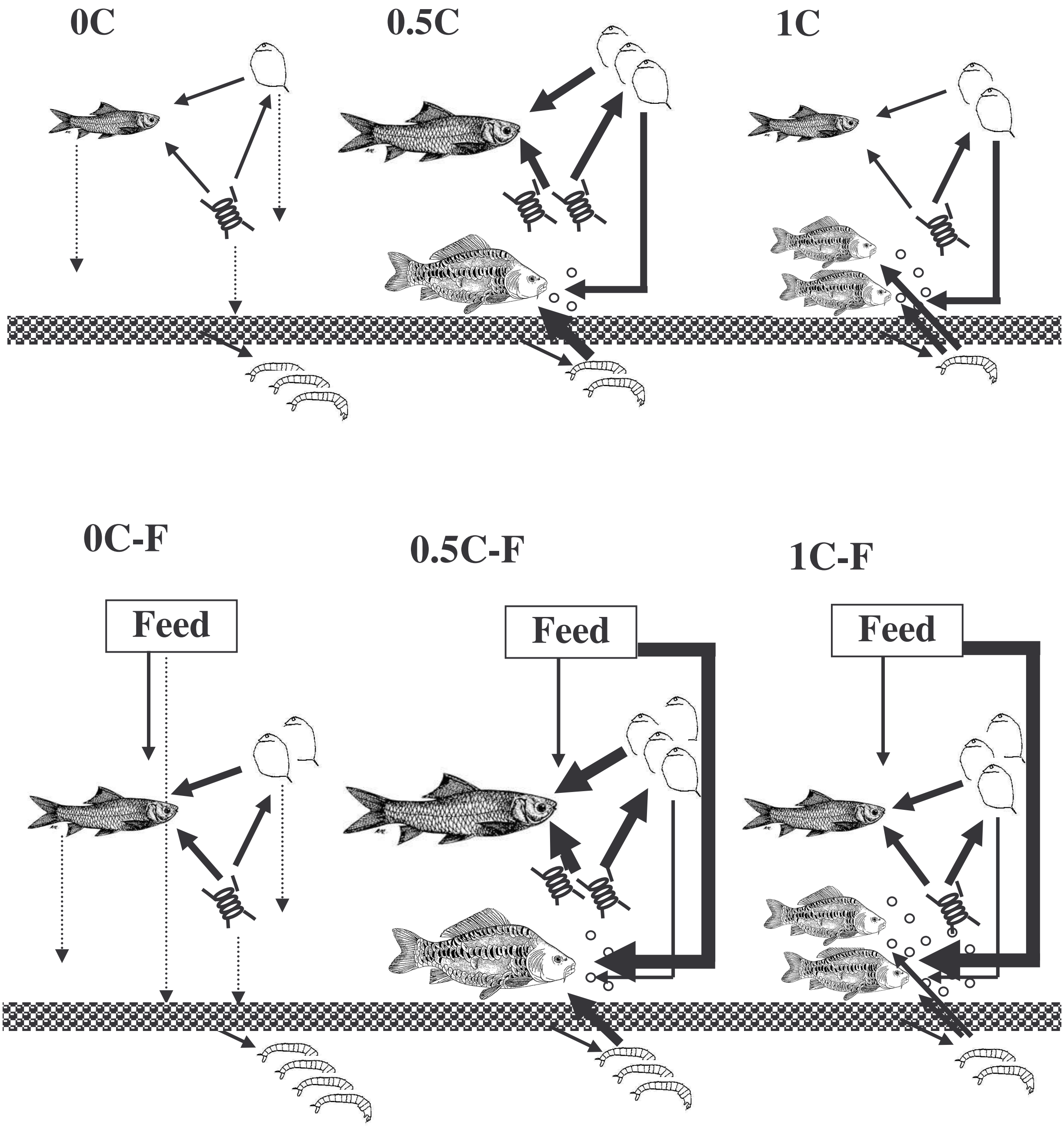

Figure 3. Conceptual model of major different trophic relations in differents treatments. Width of arrows towards similar direction among different treatments represent relative importance of effects. Size of the same species of fish represents growth performance. Arrows with dotted lines indicate sedimentation of particles on the bottom, but are not shown in all treatments to keep clarity.

the nitrogen and 19% of the phosphorus (Siddique et al., 1999) in the artificial feed are retained by the fish. Another 14% of the nitrogen and 21% of the phosphorus dissolves and is used by phytoplankton (Neori and Krom, 1991). The remaining nitrogen and phosphorus mainly stimulates bacteria, fungi and protozoa production, which in turn may be consumed by zooplankton (Tang, 1970; Langis et al., 1988). In the present study, artificial feed significantly affected the availability of food. A relatively higher quantity of zooplankton and benthic macroinvertebrate biomass in the pond were observed in treatments with feed than in those without feed, but feed had no significant effect on phytoplankton biomass (Figure 3, comparison between upper and lower section). The possible reason may be that the higher quantities of zooplankton quickly harvested phytoplankton resulting in no significant effect of feed on phytoplankton biomass. Our results partially agree with those of Moriarty (1986) and Spataru et al. (1980). Moriarty (1986) observed higher meiofauna biomass in fed ponds than in those that did not receive feed. Spataru et al. (1980) found that zoobenthos was three times higher in ponds with artificial feed than in ponds without it.

Effect on gut content

Food ingestion in fish is highly variable and depends on a variety of factors, including availability of the different food items and feed, species combination and their interactions. Fishes can consume different food organisms in different amounts under various species combinations and densities (Milstein and Svirsky, 1996). Proper association of fish species may help to develop synergism. Stocking density influences individual food availability with high densities causing preferred foods to become depleted (Milstein, 1992). This might lead to shifts from planktivory to piscivory as with common carp eating tilapia fry at high density when there are insufficient other natural foods available (Spataru and Hepher, 1977). In the present study, in the presence of common carp more natural food was available, enhancing food intake. At a density of 1 common carp m^{-2} natural foods became limiting, affecting food intake of both rohu and common carp. This was not the case at a density of 0.5 common carp m^{-2} (Figure 3).

Rohu ingested more phytoplankton and zooplankton in treatments with feed than in non-fed treatments. It preferred phytoplankton and zooplankton above artificial feed but the artificial feed had a fertilizing effect that increased phytoplankton and zooplankton abundance. Rohu showed an interesting shift in feeding behaviour in the presence of artificial feed. It ingested twice as much phytoplankton than zooplankton in non-fed treatments. In contrast, in the presence of artificial feed rohu ingested 1.3 times more zooplankton than phytoplankton, although in both treatments more phytoplankton was available than zooplankton. In the presence of artificial feed, this shifting food habit of rohu from phytoplankton to zooplankton indicates that zooplankton is a more preferable food item than phytoplankton. This result in a way agrees with Miah et al. (1984), who reported that zooplankton is a preferable food item than phytoplankton for rohu fry.

A nearly opposite trend was observed in the food preference of common carp. Actually, common carp is an omnivorous fish (Merla, 1969; Chapman and Fernando, 1994), feeding on everything available in the pond but mainly on artificial feed, zooplankton and benthic organisms and detritus (Hepher and Pruginin, 1981; Man and Hodgkiss, 1981; Milstein et al., 1991; Liang et al., 1999). In the present study, it was shown that in non-fed ponds, common carp preferred benthic macroinvertebrate, followed by zooplankton and phytoplankton. In fed ponds it preferred artificial feed. Spataru et al. (1980) reported common carp ingesting artificial feed, benthic macroinvertebrate and zooplankton. In another study, Spataru et al. (1983) reported that the most frequent items of natural food in the guts of common carp consisted of insect larvae and pupae, oligochaetes and some zooplankton species. The largest weight of natural food consisted of chironomid larvae and pupae, oligochaetes and cladocerans. Schroeder (1983) observed that in fed ponds 40% of common carp production is based on the artificial feed and 60% is based on natural foods.

The shifting food habit of common carp most likely hampers the increase in phytoplankton biomass in the fed treatments. In presence of artificial feed, common carp shifts preference from zooplankton and benthic macroinvertebrate to artificial feed, hence more zooplankton and benthic macroinvertebrate prevent algal biomass to increase even with more nutrients available (O'Brien and deNoyelles Jr., 1974), resulting in the lack of effect of artificial feed on algal biomass. However, this shifting food habit of common carp most likely increased zooplankton biomass, which facilitated rohu to ingest more zooplankton in fed treatments.

Effect on fish growth parameters

The growth performances of rohu and common carp were affected by common carp density. Rohu growth increased when common carp was present, but the effect was stronger at a density of 0.5 common carp m^{-2} than 1 common carp m^{-2}. Rohu production and total production increased almost twice in the presence of 0.5 common carp m^{-2}. Growth performance of rohu was more or less similar in treatments without common carp and treatments with 1 common carp m^{-2}. This concurs with Wahab et al. (2002), who reported a 1.6 times higher rohu yield in the presence common carp. When fish density is high competition for food becomes important. Forester and Lawrence (1978) found that high density of common carp decreased standing crop of bluegill *Lepomis macrochirus* (Rafinesque) through food competition, which caused the blugill to eat their own eggs. Hepher et al., (1989) reported positive effects at the lower density of silver carp *Hypophthalmichthys molitrix* (Valenciennes) and negative effects at the higher density of silver carp on its own and other fish species performances, including common carp.

CONCLUSION

Strong synergistic effects in terms of availability of food, food intake, growth and production were obtained in rohu ponds with 0.5 common carp m^{-2}. These effects nearly disappeared in treatments with 1 common carp m^{-2} compared to common carp-free controls. As expected, feed addition resulted in higher growth performance of rohu and common carp. So, polyculture with 0.5 common carp m^{-2} and 1.5 rohu m^{-2} with artificial feed was the best combination. Rohu shifted its feeding habit from phytoplankton to zooplankton in the presence of artificial feed. Common carp preferred benthic macroinvertebrate, followed by zooplankton and phytoplankton in non-fed ponds, but shifted to artificial feed when available. Natural food availability was quantified and fish growth and production measured. However, the relation between food availability and growth is not linear, so the underlying mechanisms need further study. In this context, a lot of additional information could be gained from direct observational studies focusing on food selectivity, feeding behaviour and social interactions in mono or polyculture systems.

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Chapter 4

Effects of common carp *Cyprinus carpio* (L.) addition and feeding on pond water quality and nutrient accumulation in rohu *Labeo rohita* (Hamilton) ponds

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ABSTRACT

The effects of introducing benthivorous common carp *Cyprinus carpio* (L.) and of adding of artificial feed on water quality and nutrient accumulation efficiency in rohu *Labeo rohita* (Hamilton) ponds studied in fertilized aquaculture ponds. The study was carried out at Bangladesh Agricultural University, Bangladesh. All ponds were stocked with 1.5 rohu m^{-2}. Treatments were executed in triplicate and incorporated: rohu alone with and without feed; rohu plus 0.5 common carp m^{-2} with and without feed; and rohu plus 1 common carp m^{-2} with and without feed. The duration of the experiment was four and half months. Addition of common carp greatly affected the water quality and nutrient accumulation efficiency at the different trophic levels. The overall highest nitrogen and phosphorus concentration were observed in treatments with 0.5 common carp m^{-2} and followed by treatments with 1 and 0 common carp m^{-2}. Nevertheless, all water quality parameters remained within favourable ranges for fish growth. The overall highest N and P accumulations in fish, phytoplankton and zooplankton were observed in treatments with 0.5 common carp m^{-2}, followed by treatments with 1 common carp m^{-2} and subsequently treatments without common carp. Accumulation efficiency in detritus was relatively higher in treatments without common carp than in those with it. More nutrients accumulated in sediments in treatments without common carp compared to those with common carp. Higher N and P accumulation efficiencies in fish, phytoplankton, zooplankton and detritus were observed in non-fed ponds than in fed ones, while the opposite was observed for nutrient accumulation in the sediment.

Keywords: Water quality; Nutrient accumulation; Artificial feed; Pond; Rohu; Common carp.

INTRODUCTION

Water quality is strongly influenced by pond management including culture species combinations and their densities, and the quality and quantity of nutrient input (Milstein, 1993; Diana et al., 1997). The mechanisms through which fish species influence water quality depend largely on nutrient-mediated effects, i.e. algae takes N and P nutrients from the water column during photosynthesis and planktivorous fish can improve the water quality by reducing algal biomass as well as water turbidity; on the other hand, benthivorous fish species (e.g., bream *Abramis brama* (L.), mrigal *Cirrhinus cirrhosus* (Bloch) and common carp *Cyprinus carpio* (L.)) stir the pond bottom and bring nutrients into the water column (Breukelaar et al., 1994; Cline et al., 1994), directly accelerating photosynthesis and indirectly nutrients accumulation efficiency in natural foods including phyto- and zooplankton, and benthos. Some studies discussed the effects of introducing planktivorous (e.g., Lyche et al., 1990; Sanni and Waervagen, 1990; Jeppesen et al., 1990) and benthivorous (e. g., Tatrai et al., 1997; Lougheed et al., 1998; Zambrano et al., 2001) fish on water quality. Most of these studies concentrate on the effects of fishes on turbidity by reducing algal biomass or by increasing inorganic suspended solids concentrations in the water column. Very little attention has been paid on the interaction effects of benthivorous and planktivorous species on water quality. Moreover, this interaction should be different in fed and unfed systems. So far, no study combined the effects of stocking different densities of benthivorous fish and artificial feed on water quality in ponds with planktivorous fishes.

In ponds, nutrients are supplied as fertilizer or feed. In ponds receiving protein-rich pellets only 11-35% of the supplied N and 13-36% of P were retained in fish biomass (Table 1). A large fraction of the unused N and P accumulated in the system affecting water quality in the overlaying water column. Sediment can store 100-1000 times more nutrients than water (Biro, 1995). Proper resuspension can transfer these nutrients back into the water column. However, such resuspension not only affects water quality but also the fraction of input nutrient accumulated in benthos, zooplankton, phytoplankton and also culture animals. Fast mineralization and utilization of nutrients by culture organisms will improve water quality and natural food availability, improving the apparent feed conversion ratios. Information on nutrient accumulation efficiencies in culture organisms is mainly based on monoculture studies with hybrid tilapias (*Oreochromis mossambicus* (Peters) × *O. niloticus* (L.); *O.*

niloticus × *O. aureus* (Steindachner)), channel catfish *Ictalurus punctatus* (Rafinesque), and gilthead seabream *Sparus aurata* (L.) supplied with artificial feed. If high densities of benthivorous would be added to these monoculture systems the resulting increase in turbidity would affect primary productivity and the amounts of nutrients accumulated in natural foods, including phytoplankton, zooplankton and benthos. The nature of these effects is still poorly understood. Therefore, the aim of this study was to monitor the effects of stocking different densities of the benthivorous common carp in ponds planktivorous rohu on water quality and the amount of nutrients retained by cultured fishes and natural foods. Artificially fed and non-fed ponds were compared.

Table 1. Reported accumulation efficiency (%) of fish, phytoplankton, water and sediment.

Types	Accumulation (%)		System	References
	N	P		
Channel catfish (*Ictalurus punctatus*)	26.8	30.1	Earthen pond	Boyd, 1985.
Tilapia (*Oreochromis niloticus*)	18-21	16-18	Earthen ponds	Green and Boyd, 1995.
Hybrid tilapia (*O. mossambicus* × *O. niloticus*)	17.5	-	Earthen pond	Acosta-Nassar et al., 1994.
Gilthead Seabream (*Sparus aurata*)	26	21	Marine pond	Neori and Krom, 1991.
Gilthead Seabream (*S. aurata*)	29	36	Marine pond	Krom et al., 1985.
Gilthead Seabream (*S. aurata*)	21	26	Marine pond	Krom and Neori, 1989.
Rainbow trout (*Oncorhynchus mykiss*)	18.9	13.2	Circular tank	Foy and Rosell, 1991.
Carp	11	32	Earthen pond	Avnimelech and Lacher, 1979.
Polyculture	11-16	-	Earthen ponds	Schroeder et al., 1990.
Shrimp (*Penaeus monodon*)	24	13	Shrimp pond	Briggs and Funge-Smith, 1994.
Shrimp (*P. monodon*)	22	-	Shrimp farm	Jackson et al., 2003.
Phytoplankton	0.74	-	Earthen pond	Acosta-Nassar et al., 1994.
Particulate matter	46	50	Marine pond	Krom and Neori, 1989.
Water	10.4	-	Earthen pond	Acosta-Nassar et al., 1994.
Water	14	21	Marine pond	Neori and Krom, 1991.
Water	13	22	Marine pond	Neori and Krom, 1991.
Detritus	10	17	Marine pond	Krom and Neori, 1989.
Sediment	70	35-40	Earthen ponds	Green and Boyd, 1995.
Sediment	31	84	Shrimp pond	Briggs and Funge-Smith, 1994.
Sediment	11	15	Marine pond	Neori and Krom, 1991.
Sediment	14	-	Shrimp farm	Jackson et al., 2003.
Loss to atmosphere	0.92	-	Earthen pond	Acosta-Nassar et al., 1994.

MATERIALS AND METHODS

Experimental design

The Experiment was carried out at the Fisheries Faculty Field Laboratory, Bangladesh Agriculture University (BAU), Mymensingh, in 18 earthen ponds of 100 m^2 and 1.2 m depth. All ponds were stocked on March 15, 2003 with 1.5 rohu m^{-2}. The experiment had a 3×2 factorial design with three levels of common carp (CC) density (0, 0.5 and 1.0 m^{-2}) as first factor and two levels of artificial feed (with and without feed) as second factor. Each treatment was executed in triplicate. The duration of the experiment was 4.5 months.

Pond preparation, fish stocking and management

Prior to fish stocking, ponds were drained to eradicate all remaining weeds and animals and to repair embankments and slopes. Agricultural lime ($CaCO_3$) was applied at 250 kg ha^{-1} to all ponds. Ponds were filled with ground water from an adjacent deep tube-well 7 days before fertilization with 1,250 kg ha^{-1} decomposed cow manure; 31 kg ha^{-1} urea and 16 kg ha^{-1} triple super phosphate (TSP). Thereafter the same amounts of fertilizers were applied fortnightly throughout the study period. Urea contained 46% nitrogen; TSP 20% phosphorus; and decomposed cow manure 0.33% nitrogen, 0.09% phosphorus and 79% moisture. Rohu (mean individual weight of 20.3-21.1g) and common carp (20.6-21.4 g) fingerlings collected from a nearby nursery were released in the afternoon into the ponds, taking care to gradually acclimatize the fishes to pond conditions. Pelleted artificial feed (ingredients: fish meal, rice bran, mustard oilcake and vitamin premix) containing 5.36% nitrogen and 0.73% phosphorus, was supplied daily at 15 g $kg^{-0.8}$ day^{-1} starting the day after stocking until harvesting. Feeding rates were adjusted monthly after weighing minimum 20% of the number of fishes stocked per pond. Because the highest fish growth rates were achieved in 0.5 CC m^{-2} treatments, these treatments received higher amounts of artificial feed than the 1 CC m^{-2} treatments. The smallest amount was fed to CC-free ponds.

Water quality analysis

A series of physico-chemical parameters (temperature, dissolved oxygen (DO), pH, transparency, total alkalinity, nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), total ammonia nitrogen (TAN), total nitrogen (TN), phosphate phosphorus (PO_4-P), total phosphorus (TP), chlorophyll a (Chl-a) and total suspended solid (TSS)) were determined fortnightly between 9.00 and 10.00 am, starting the day before fish stocking until harvesting. TN and TP in water, plankton and detritus were also analyzed before fertilization started. Temperature was measured with a centigrade thermometer, DO by the Winkler titration method (Stirling, 1985), transparency with a Secchi disc, pH with a Jenway 3020 pH meter. Total alkalinity was determined by the titrimetric method (Stirling, 1985). TAN and PO_4-P were analyzed spectrophotometrically (Milton Roy Spectronic, model 1001 plus; Stirling, 1985). NO_2-N (diazotization), NO_3-N (cadmium reduction), TP (acid persulphate digestion method) and total Kjeldahl-N were determined according to APHA (1998). TN was determined as the sum of NO_2-N, NO_3-N and Kjeldahl-N. TSS was determined according to Stirling (1985). Chlorophyll-a was analyzed spectrophotometrically after acetone extraction following Boyd (1979),

$$\text{Chlorophyll-a } (\mu g\ L^{-1}) = 11.9(E_{665}-E_{750})V/L\times1000/S,$$

where E_{665} = optical density of the sample measured at 665 nm; E_{750} = optical density at 750 nm; V = acetone volume used (ml); L = volume of sample filtered (ml) and S = length of light path in the spectrophotometer (cm).

Plankton and detritus sampling

Qualitative and quantitative determinations of plankton were done fortnightly. For this, a measured volume of water (minimum 20 L, maximum 100 L) was collected with a Niskin water sampler from 10-20 different locations in each pond. Water samples were passed first through a 60-µm mesh zooplankton net and subsequently through a 10-µm phytoplankton net. Collected zooplankton and phytoplankton were placed on a pre-weighed and labelled aluminum foil for further analysis. Detritus samples were collected fortnightly with an Ekman dredge. Detritus included non-living organic matter and living organism collected after sieving sediments through a 250-µm mesh sieve. Composite mud samples were made from three

Ekman-dredge samples collected from different sites in each pond. Detritus collected after sieving was placed on a pre-weighed and labeled piece of aluminum foil for further analysis.

Fish harvesting and proximate composition analysis of feed, fish, plankton and detritus

At the end of the experiment, ponds were drained, all fishes were harvested and weighed individually with a precision of 0.1g. Proximate composition analysis (moisture, lipid, ash, TN and TP) of feed, fish, plankton and detritus were done in the feed technology laboratory, Bangladesh Agricultural University, Bangladesh. Samples were oven-dried (Memmert, model UM/BM 100-800) at 105 ^{0}C for 24 hours to determine moisture content (APHA, 1998) and burned in a muffle furnace (550 ^{0}C for 6 hours) to determine ash content (APHA, 1998). Fat content was determined using the Soxhlet apparatus. Standard methods (AOAC, 1984) was followed to estimate TN (Kjeldahl method) and TP.

Data calculations and analysis

The total input of N and P were calculated as the combined inputs of urea, TSP, cow manure and artificial feed. N and P accumulation (%) in fish, phytoplankton, zooplankton and detritus were calculated based on biomass. Phytoplankton and zooplankton biomass were calculated as their concentration multiplied by the pond water volume. Detritus biomass was calculated as its concentration multiplied by the pond area. N and P accumulation were calculated as,

$$\text{N or P accumulation (\%)} = ((B_f \times N_f) - (B_i \times N_i)) \times 100/\ N_{in},$$

where B_f = final biomass; N_f = final nutrient (N or P) concentration; B_i = initial biomass; N_i = initial nutrient (N or P) concentration; N_{in} = total nutrient (N or P) input. The initial biomass of plankton and detritus was determined before the first fertilization. Nutrient accumulation in the sediment was calculated indirectly as,

$$\text{N or P accumulation in the sediment (\%)} = 100 - (R_f + R_p + R_d + R_w),$$

where R_f = nutrient accumulation by fish; R_p= nutrient accumulation by plankton; R_d = nutrient accumulation by detritus; R_w = nutrient accumulation in the water column. Water quality, nutrient inputs and nutrient accumulation parameters were analyzed using the statistical package SAS, version 8. A two-way split plot ANOVA was performed with common carp densities and artificial feed addition as the main factors and time as the sub-factor (Gomez and Gomez, 1984). If a main effect was significant, the ANOVA was followed by Tukey mean multi-comparison test at $P<0.05$ level of significance. All percentage data were arcsine transformed before analysis and checked for normal distribution. Because of the scope of the present paper, we will discuss only the effects of main factors. The sub-factor time and the interactions between time and the main factors will be presented in a separate article.

RESULTS

Effects of common carp and artificial feed on water quality

The ANOVA and Tukey test results of the water quality parameters are shown in Table 2. The presence of common carp (CC) significantly ($P<0.01$) affected all water quality parameters except temperature and NO_2-N. DO concentration decreased with increasing common carp density. Relatively lower pH values were observed in treatments with than without CC. Total alkalinity was higher in the treatment without CC ($P<0.01$) while treatments with 0.5 and 1 CC m^{-2} were similar ($P>0.05$).

The NO_2-N concentration was always very low in all treatments compared to NO_3-N and TAN concentrations. NO_3-N, TAN and TN concentrations behaved similarly. Higher concentrations were observed in treatments with than in those without CC ($P<0.001$) but there was no difference between the treatment with 0.5 and 1 CC m^{-2} ($P>0.05$). Higher PO_4-P and TP concentrations were observed in treatments with CC whereas these concentrations in treatments with 0.5 CC were higher than in treatments with 1 CC m^{-2} ($P<0.001$). Secchi disk transparency was higher in CC-free ponds than in ponds with 1 CC m^{-2}, which in turn was higher than with 0.5 CC m^{-2} ($P<0.001$). Opposite trends were observed for Chlorophyll-a (Chl-a) and TSS. Higher Chl-a and TSS concentrations were observed with 0.5 CC m^{-2} than with 1 CC m^{-2}, and subsequently 0 CC m^{-2} ($P<0.001$).

The Application of artificial feed influenced all parameters except water temperature and NO_2-N ($P>0.05$) (Table 2). Feeding lowered DO and total alkalinity in water. A similar, but weak, trend was observed for pH. All nitrogenous and phosphorus compounds reacted similarly to feeding, showing higher concentrations in fed ponds, except NO_2-N which was similar in fed and non-fed ponds. Feeding lowered Secchi disc visibility. The concentrations of Chl-a and TSS were higher in fed ponds. The interaction between artificial feed and CC had significant effects on most water quality parameters except temperature, total alkalinity, NO_2-N and Secchi disc visibility (Table 2 and Figure 1). Artificial feed increased the effect of CC on most of the water quality parameters (Table 2).

Table 2. Effects of common carp and supplementary feed on different water quality parameters based on two-way ANOVA.

Variable	ANOVA Significance (*P* value)			Means (Tukey test)				
				Common carp density			Feed	
	CC	Feed	CC×Feed	0	0.5	1	With	No
Temperature (^{0}C)	NS	NS	NS	27.6	27.6	27.5	27.6	27.6
DO (mg L^{-1})	***	***	**	7.1^a	6.4^b	6.1^c	6.3^b	6.7^a
pH range	-	-	-	7.2-8.4	6.5-8.3	6.5-8.2	6.5-8.3	6.8-8.4
Tot. Alk. (mg L^{-1})	**	**	NS	142.0^a	135.8^b	136.2^b	135.5^b	140.5^a
NO_2-N (mg L^{-1})	NS	NS	NS	0.030	0.028	0.027	0.031	0.027
NO_3-N (mg L^{-1})	***	***	***	0.78^b	0.96^a	0.95^a	0.96^a	0.84^b
TAN (mg L^{-1})	***	***	***	0.25^b	0.32^a	0.31^a	0.33^a	0.26^b
TN (mg L^{-1})	***	***	*	1.52^b	1.93^a	1.81^a	1.88^a	1.62^b
PO_4-P (mg L^{-1})	***	***	***	0.74^c	1.07^a	1.00^b	1.01^a	0.87^b
TP (mg L^{-1})	***	***	***	1.28^c	1.67^a	1.58^b	1.57^a	1.44^b
Transparency (cm)	***	***	NS	26.61^a	18.17^c	20.79^b	20.74^b	22.97^a
Chl-a (µg L^{-1})	***	**	*	129.84^c	153.76^a	136.94^b	143.38^a	136.98^b
TSS (mg L^{-1})	***	***	**	1.06^c	2.96^a	2.58^b	2.25^a	2.15^b

CC = common carp density; Feed = artificial feed addition; CC×Feed = interaction of common carp density and feed; 0 = treatments without common carp; 0.5 = treatments with 0.5 common carp m^{-2}; 1 = treatments with 1 common carp^{-2}; With = treatments with feed; No = treatments with no feed. Mean values in the same row with no superscript in common differ significantly ($P<0.05$). If the effects were significant, ANOVA was followed by Tukey mean multicomparison test. *$P\leq0.05$; **$P<0.01$; ***$P<0.001$; NS, not significant.

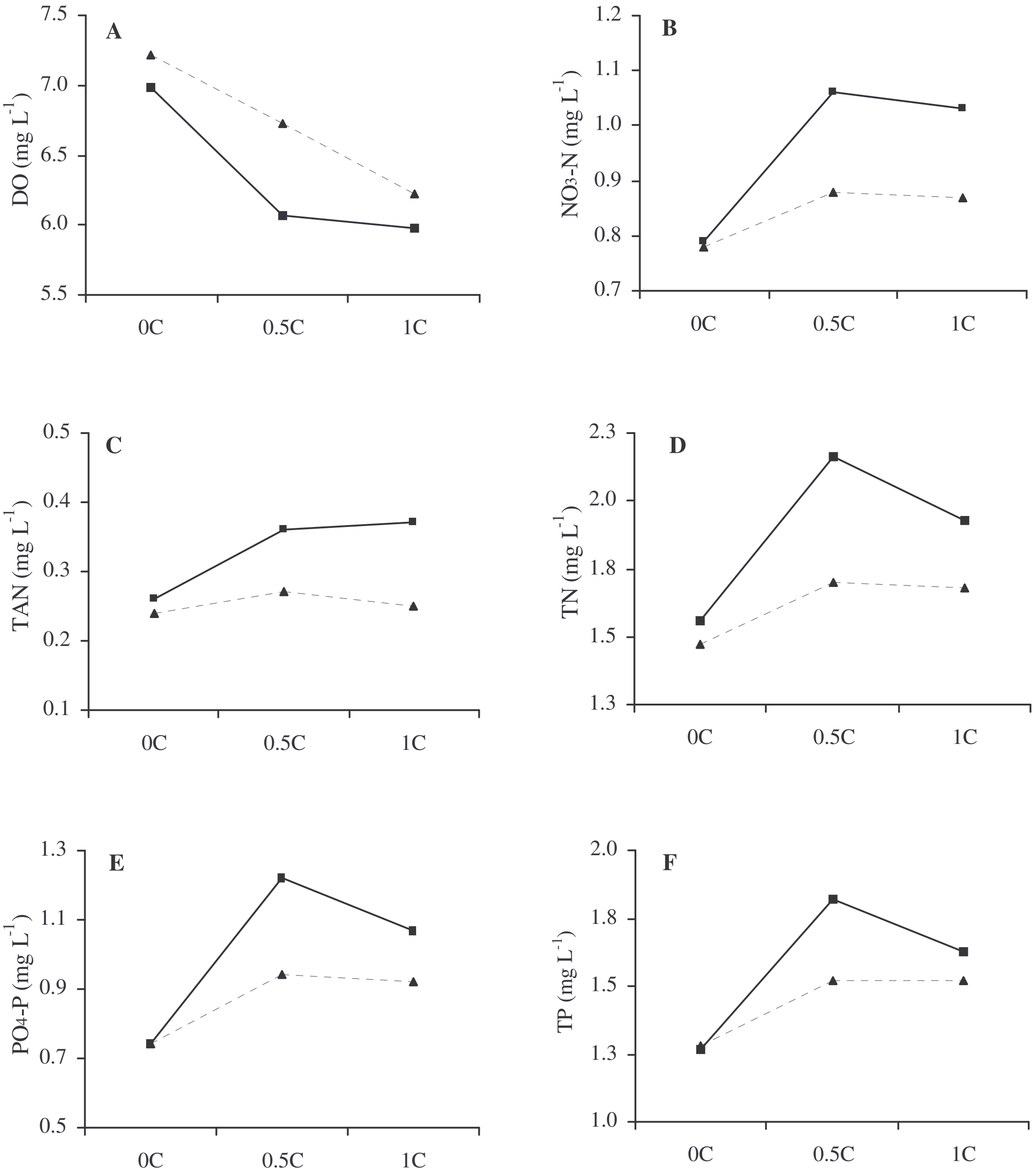

Figure 1. Interaction effects of common carp density and artificial feed on the DO (A), NO_3-N (B), TAN (C), TN (D), PO_4-P (E) and TP (F) concentration in the water. 0C = treatments without common carp, 0.5C = treatments with 0.5 common carp m^{-2} and 1C = treatments with 1 common carp m^{-2}. Solid and dotted lines indicate treatment with and without feed, respectively.

Effects of common carp and artificial feed on N and P accumulation

The presence and density of CC showed significant effects (*P*<0.001) on the N and P accumulation in fish (Table 3). The N accumulation was 1.9 times and 1.6 times higher in treatments with 0.5 and 1 CC m^{-2}, respectively, compared to the treatments without CC. Similarly, the P accumulation was 2 times higher in treatments with than in treatments without CC (*P*<0.001), but there was no significant difference between treatments with 0.5 and 1 CC m^{-2} (*P*>0.05). N and P accumulation in phytoplankton showed almost similar pattern to N and P accumulation in fish. Accumulation was around 1.5 times higher in ponds with CC (*P*<0.01), and was not affected by CC density (*P*>0.05). N and P accumulation by zooplankton ranged between 1 and 2%. More N was retained in zooplankton in 0.5 CC m^{-2} ponds (*P*<0.05) than in ponds without or with 1 CC m^{-2}, accumulation in the latter ponds being similar to CC-free ponds (*P*>0.05). P accumulation in zooplankton was not influenced by the presence or density of common carp (*P*>0.05).

Only about 2% N was retained in detritus compared to >16% P. N accumulation by detritus was not influenced by CC presence or density (*P*>0.05), whereas less P accumulated in detritus in the presence of CC (*P*<0.01), similarly at both CC densities. More N and P remained in the water column in the presence of CC (*P*<0.05), where N accumulation was similar in the treatments with 0.5 and 1 CC m^{-2} but P accumulation was 1.3 times higher with 1 CC m^{-2} than with 0.5 CC m^{-2}. The percentage of N and P accumulation in the sediment was higher in CC-free ponds (*P*<0.001). N accumulation in the sediment was higher with 1 CC m^{-2} than with 0.5 CC m^{-2} (*P*<0.001), whereas P accumulation in the sediment was not affected by CC density (*P*>0.05).

Feeding strongly affected (*P*<0.001) N and P accumulation in fish, phytoplankton, zooplankton, detritus, water column and sediments. Higher nutrient percentages were retained in fish (N, 1.9 times; P, 1.5), phytoplankton (N, 2.7; P, 2.1), zooplankton (N, 2.7; P, 2.1), detritus (N, 3; P, 2) and in the water column (N, 2; P, 1.5) in non-fed ponds. An opposite trend was observed for N and P accumulation in the sediment. On the average, 1.5 times more N and 2.6 times more P were lost in fed ponds. Significant interactions were found between CC density and feeding for N accumulation in fish, phytoplankton, zooplankton and sediment, but

Table 3. Effects of common carp and supplementary feed on different retention parameters based on two-way ANOVA.

Variable	Significance (*P* value)			Means (Tukey test)				
				Common carp			Feed	
	CC	Feed	CC×Feed	0	0.5	1	With	No
Accumulation of nitrogen (%)								
Fish (Rohu plus Common carp)	***	***	*	13.4^{c}	25.8^{a}	21.3^{b}	14.1^{b}	26.2^{a}
Phytoplankton	***	***	**	6.8^{b}	9.3^{a}	9.5^{a}	4.6^{b}	12.4^{a}
Zooplankton	*	***	***	1.8^{b}	1.9^{a}	1.8^{b}	1.0^{b}	2.7^{a}
Detritus	NS	***	NS	2.1	1.8	2.0	1.0^{b}	2.9^{a}
Water	*	***	*	3.5^{b}	5.0^{a}	5.4^{a}	3.1^{b}	6.2^{a}
Accumulation in the sediment	***	***	**	72.4^{a}	56.2^{c}	60.2^{b}	76.2^{a}	49.6^{b}
Accumulation of phosphorus (%)								
Fish (Rohu plus Common carp)	***	***	*	10.9^{b}	22.7^{a}	21.9^{a}	14.8^{b}	22.3^{a}
Phytoplankton	**	***	*	3.1^{b}	4.8^{a}	5.1^{a}	2.8^{b}	5.9^{a}
Zooplankton	NS	***	NS	1.1	1.1	1.1	0.7^{b}	1.5^{a}
Detritus	**	***	NS	23.8^{a}	19.8^{b}	16.5^{b}	13.2^{b}	26.8^{a}
Water	***	***	NS	14.2^{c}	18.5^{b}	24.2^{a}	15.1^{b}	22.8^{a}
Accumulation in the sediment	***	***	*	46.9^{a}	33.1^{b}	31.1^{b}	53.4^{a}	20.7^{b}

CC = common carp density; Feed = artificial feed addition; CC×Feed = interaction of common carp density and feed; 0 = treatments without common carp; 0.5 = treatments with 0.5 common carp m^{-2}; 1 = treatments with 1 common carp^{-2}; With = treatments with feed; No = treatments with no feed. Mean values in the same row with no superscript in common differ significantly ($P<0.05$). If the effects were significant, ANOVA was followed by Tukey test. *$P \leq 0.05$; **$P<0.01$; ***$P<0.001$; NS, not significant.

not for detritus. In the case of P accumulation, significant interactions were found for fish, phytoplankton and sediment but not for zooplankton, detritus and water column (Table 2 and Figure 2).

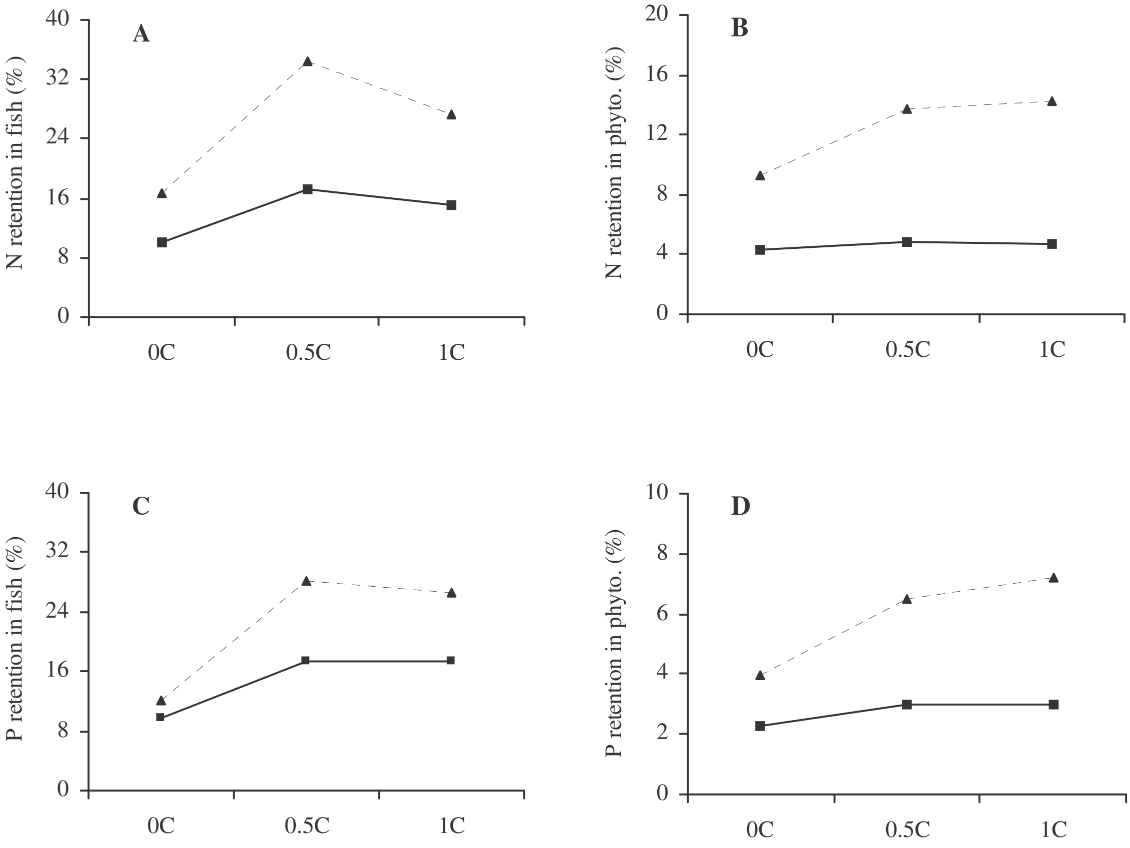

Figure 2. Interaction effects of common carp density and artificial feed on the nitrogen retention efficiency in fish (A) and phytoplankton (B), phosphorus retention efficiency in fish (C) and phytoplankton (D). 0C = treatments without common carp, 0.5C = treatments with 0.5 common carp m^{-2} and 1C = treatments with 1 common carp m^{-2}. Solid and dotted lines indicate treatment with and without feed, respectively.

DISCUSSION

Effects of common carp and artificial feed on water quality

In general, ponds were more eutrophic with higher nutrient concentrations in treatments with 0.5 common carp m^{-2}, but all water quality parameters remained within ranges allowing high fish growth rates. Almost all water quality parameters were affected by the presence of benthivorous CC. Stirring of sediments by benthivorous fishes has two effects: (1) it increases diffusion rates across the sediment-water interface (Hohener and Gachter, 1994), and (2) it increases aerobic decomposition by aerating anaerobic sediments (Graneli, 1979; Beristain, 2005). These two effects enhance the ammonia and phosphorus flux from the sediments to the water column (Hargreaves, 1998). In the present study, stirring by CC resulted in higher water column concentrations of N and P species (except NO_2-N that is very quickly removed by nitrifying bacteria and algae) compared to CC-free ponds (Ritbo et al., 2004). In turn, the increased nutrient availability resulted in increased primary production, as indicated by a higher concentration of Chl-a and TSS and lower water transparency in ponds with common carp. Carbon dioxide is released during decomposition (Moriarty, 1997), and might have contributed to the lower pH and alkalinity observed in CC ponds compared to CC-free ponds. DO concentrations decreased with increasing CC density, possible due to (1) additional respiration by CC and/or (2) higher aerobic bacterial decomposition rates caused by CC sediment stirring (Andersson et al., 1988).

For N-species, the concentrations did not differ between 0.5 and 1 common carp m^{-2}. Larger amounts of particulate nitrogen are released by larger biomasses of fish and plankton present (Kaushik, 1980; Dolan, 1997) and since a higher biomass of fish and plankton was present in treatments with 0.5 than in those with 1 CC m^{-2} (Rahman et al., 2006), it might be expected that more particulate nitrogen is released in the ponds with 0.5 than 1 CC m^{-2}. From particulate matter settling in the bottom, NH_4-N is released. The liberation efficiency of NH_4-N and NO_3-N from sediment to water might be comparatively less in treatment with 0.5 CC than 1 CC m^{-2} because of less stirring. So, high production and less liberation from sediment might have resulted in a similar concentration in treatments with 0.5 and 1 CC m^{-2}. However, the TN concentration was higher in treatments with 0.5 than in those with 1 CC m^{-2} in fed ponds (Figure 1-D). This suggests that in the presence of artificial feed, the abundance of particulate nitrogen (fish faeces, plankton and other organic matter, etc.) was higher in treatments with 0.5 than 1 CC m^{-2}.

At a redox potential above 200 mV, a portion of the newly transformed soluble phosphorus (PO_4-P) will precipitate forming phosphate-rich inorganic particles (e.g., with iron as iron (III) phosphate) (Holdren and Armstrong, 1980; Bostrom et al., 1988; Boyd, 1995). This precipitation rate might have been higher in treatments with 1 than in those with 0.5 CC m^{-2}, because sediment stirring increased with increasing CC density. As a result, the concentrations of soluble phosphorus and DO in the water column were lower in treatments with 1 than in those with 0.5 CC m^{-2}. Another plausible reason explaining the higher phosphorus concentration is the higher biomass of fish, zooplankton and benthic macroinvertebrate in treatments with 0.5 than with 1 CC m^{-2}, all of which release phosphorus (Gallepp, 1979; Brabrand et al., 1990; Lupatsch and Kissil, 1998). Higher PO_4-P concentration resulted in higher Chl-a (as also recorded by Yusoff and McNabb, 1997) and TSS and lower water transparency in treatments with 0.5 than in those with 1 CC m^{-2}.

Artificial feed supplied an additional 132-241% N and 79-140% P to the ponds. In aquaculture, major portions of artificial feed accumulate in ponds as uneaten feed, faeces and metabolites (Daniels and Boyd, 1989; Briggs and Funge-Smith, 1994; Kibria et al., 1997). Hakanson et al. (1988) calculated that of the total P and N fed to fish, 70% P and 15% N were defecated. In this study, the higher fish biomass in fed treatments also produced more faeces. More faeces and more uneaten feed resulted in higher concentrations of nitrogenous and phosphorus compounds and more decomposition in fed treatments. As a result, DO levels, pH and alkalinity were lower in treatments with than in those without feed. Higher amounts of nitrogenous and phosphorus compounds enhanced photosynthesis, explaining the higher plankton production, higher TSS values and lower transparency in fed treatments compared to non-fed treatments (Poxton, 1991; Milstein and Svirsky, 1996; Moriarty, 1997). However, in the present study, feeding only affected water quality in presence of CC (Figure 1), which indicates that CC stimulated the mineralization of excess organic matter coming from artificial feed. This effect was more pronounced in presence of 0.5 than 1 CC m^{-2}, especially for P mineralization.

Effects of common carp and artificial feed on N and P accumulation

In the present study, N and P accumulation efficiency in fish varied within the range of 13.4-25.8% and 10.9-22.7%, respectively, which concurs with previous studies (N 10-30% and P 15-40%) by Avnimelech and Lacher (1979), Boyd (1985), Krom et al. (1985), and Edwards (1993). N and P accumulation efficiency in fish and phytoplankton were influenced by CC presence and density. CC presence enhanced sediment resuspension and denitrification (Milstein et al., 1988; Cline et al., 1994; Milstein et al., 2002). Nutrient availability in the water column also improved photosynthesis resulting in higher N and P accumulation percentages in phytoplankton. Consequently, rohu ate more plankton, resulting in higher accumulation percentages in fish. The accumulation percentages in zooplankton were similar in treatments without CC and with 1 CC m^{-2}. Possibly, excess grazing pressure on zooplankton by CC in the latter treatment removed the additional zooplankton produced.

A higher percentage of input-N accumulated in fish and zooplankton in treatments with 0.5 than in treatments with 1 CC m^{-2}. This result suggests that increasing the stocking density above 0.5 CC m^{-2} created a negative feedback on fish and plankton production through decreased water transparency and primary productivity (Rahman et al., 2006). In this study, the P accumulation efficiency in phytoplankton was relatively low (3.1-5.1%) compared to the results of Sierp and Qin (2001), who found that after 6 h of fertilization in a laboratory test in 1 L flasks, 30% of the P was assimilated in the phytoplankton. However, their study overestimated P accumulation in natural systems, because of the absence of consumers (fish and zooplankton) and sediment, as plankton dynamics in system can be largely influenced by consumer density, sediment quality and nutrient levels (Culver and Geddes, 1993; Arumugam and Geddes, 1996; Qin and Culver, 1996).

The percentage of N accumulating in detritus was very low (around 2%) but P accumulation was very high (>16%), similar to the results of Krom and Neori (1989), who estimated an accumulation of 17% P of the total input in the detritus in intensive fish ponds with circularly moving seawater. The lower percentage of P retained in detritus in the treatments with common carp may be related to a higher consumption of detritus by carp due to grazing pressure on the sediment (Riera et al., 1991; Tatrai et al., 1994; Zambrano and Hinojosa, 1999). Another reason is that common carp helps bottom nutrients to re-enter the water column, which thus accumulate less in detritus and sediment.

N accumulation in the sediment was in the range 50-76%. This result is more or less similar to the result of Acosta-Nassar et al. (1994) who reported 65% N accumulation in the sediment in a tropical semi-intensive freshwater fish culture pond. In the present study, N loss by volatilisation was not considered because it is generally very small in earthen ponds. Acosta-Nassar et al. (1994) reported only 0.92% N loss to the atmosphere. Some algae fix atmospheric N and recover the N loss by volatilization. For P, the range of accumulation in the sediment was 31-47%, which is in concordance with Thakur and Lin (2003), who reported a 39-67% P accumulation in the sediment in concrete tanks. In the present study, N and P accumulation in the sediment were reduced in presence of CC. The effect of CC density on nutrient accumulation in the sediment might be related with stirring efficiency, turbidity, plankton production and fish grazing on plankton. More research is needed to determine clear cause-effect relationships.

Fertilizer and artificial feed were the main inputs in the present study. Higher N and P inputs were applied in treatments with 0.5 CC m^{-2}, followed by treatments with 1 and 0 CC m^{-2}. In the treatments with feed, more than 50% of the N and P came from the artificial feed (57 - 71% N and 44 - 58% P). The higher the input levels of N and P, the smaller the percentages that accumulated in fish, plankton, detritus and the water column. Conversely, a higher percentage of nutrient inputs accumulated in the sediment with higher input levels of N and P. This result agrees with Siddiqui and Al-Harbi (1999), who reported increasing nutrient accumulation efficiency in fish and decreasing nutrient accumulation in the sediment with decreasing feed input. However, when looking in more detail, this result only concurs for a comparison between fed and non-fed treatments. Comparing treatments with and without CC, treatments with CC receive more nutrient inputs and showed higher accumulation levels in fish than CC-free treatments (Figure 2).

In conclusion, the effect of stocking common carp to improve nutrient cycling and food web management was considerable. Addition of common carp enhanced the release of nutrients from the sediment, stimulating phytoplankton productivity and accelerating the flux of nutrients to higher trophic levels. Stocking 0.5 CC m^{-2} had a more pronounced effect on nutrient cycling resulting in the incorporation of higher percentages of input nutrients in fish biomass. In the present experimental set-up, stocking 1 CC m^{-2} can be considered as overstocking. At this CC density fish production was lower and more nutrients accumulated in the sediment than with 0.5 CC m^{-2}.

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Chapter 5

Swimming, grazing and social behaviour of rohu *Labeo rohita* (Hamilton) and common carp *Cyprinus carpio* (L.) in tanks under fed and non-fed conditions

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ABSTRACT

In this study we quantified feeding and swimming behaviour and social interactions of rohu, *Labeo rohita* (Hamilton), in monoculture and in polyculture with various common carp, *Cyprinus carpio* (L.) densities (1 and 2 tank^{-1}), under fed and unfed conditions. We found that rohu mainly lives and feeds in the water column. It showed decreased intra-species and increased inter-species social behaviour with increasing common carp density. In the presence of common carp rohu increased grazing and swimming near the tank bottom and reduced resting time. Grazing on tank wall and tank bottom, rohu mainly benefited from zooplankton ingestion. The sum of grazing on tank wall and tank bottom appeared to be an indicator of rohu growth performance. The sum of grazing on tank wall and tank bottom was higher at lower density common carp, followed by higher density common carp and no common carp. Common carp spent around half its time close to the bottom and mainly fed on benthic macroinvertebrates if artificial feed was not supplied. Grazing on the tank bottom was an indicator of common carp growth performance in the absence of artificial feed, although it preferred artificial feed over natural food if administrated. The analysis of water quality, natural food and growth performance of rohu and common carp indicated that the circumstances in the tanks were comparable to those in the ponds and that therefore the results from this study most likely provide insight in the (trophic) behaviour of these fish species in polyculture ponds.

Keywords: Artificial feed; Behaviour; Rohu; Common carp; Grazing; Swimming.

INTRODUCTION

Pond polyculture is the main aquaculture production system in south Asia, especially in Bangladesh and India (FAO, 1997; Reddy et al., 2002). Polyculture is based on the concept of optimal natural food utilization at different trophic and spatial levels within the pond ecosystem (Milstein, 1992; Hepher et al., 1989; Yashouv, 1971). In some cases, one species enhances the production or availability of food utilized by another species, and thus increases the total fish yield per unit of area or nutrient input (Hepher et al., 1989). In Bangladesh, a variety of fish species are commonly cultured in polyculture systems. Among those, rohu (*Labeo rohita* Hamilton) is popular and fetches high prices, while common carp (*Cyprinus carpio* L., further abbreviated as CC) is a little less popular, but it grows fast and still fetches reasonable prices in local markets.

Studying the effects of addition of different densities of CC to rohu ponds on food availability, food intake, growth and production, revealed strong synergistic effects when CC density was 0.5 fish m^{-2} and rohu density was 1.5 fish m^{-2} (Rahman et al., 2006). In fact, rohu production increased 1.4 times and total pond production doubled, most likely because CC enhanced aerobic decomposition of organic matter at the pond bottom and resuspended bottom nutrients into the water column, thereby stimulating natural food production. In consequence, food availability for rohu improved in presence of CC, in part explaining its observed higher production (article in progress). However, other mechanisms (e.g., behavioural interactions between rohu and CC) might also be involved in explaining the observed synergism. It is possible that the time that rohu spent on swimming, grazing, and intra- and inter-species interactions changed in presence of CC. This might have affected rohu's food selectivity and food intake, which in turn would have influenced its growth and production.

Besides changes in species composition, addition of supplementary feed affects the availability of natural food and influences feeding behaviour, growth and production in ponds (Takamura et al., 1993; Anras et al., 2001). Application of artificial feed to ponds resulted in higher food uptake and enhanced growth and production of both rohu and CC. Rohu shifted to eating relatively more zooplankton compared to phytoplankton, when zooplankton became more available in response to feed application to ponds. CC preferred benthos, followed by zooplankton and phytoplankton in non-fed ponds, but shifted from natural foods to artificial feed when available (Rahman et al., 2006). Therefore, addition of artificial feed may have had an influence on the feeding behaviour of rohu and CC. Moreover, swimming, grazing and social behaviour, as well as inter-species interactions may also have played a role in the observed effects of artificial feed addition.

The feeding niche of rohu in aquatic ecosystems is still not clear. Some authors consider rohu a water column feeder (Dewan et al., 1991; Wahab et al., 1994; Azim et al., 2004), others a surface feeder (Hora, 1944) and still others both a surface and water column feeder (Das and Moitra, 1955). These conclusions were mostly based on gut content analysis (Dewan et al., 1977) and not on behavioural observations. Similarly, it is well accepted that CC is a bottom feeder (Spataru et al., 1983; Chow-Fraser, 1999; Sidorkewicj et al., 1999), but no study provides accurate information about the behaviour of CC in ponds. Behavioural studies in ponds are difficult due to high turbidity and, in consequence, low visibility. Nevertheless, direct observation can provide important information on grazing and swimming behaviour and social interactions (Houlihan et al., 2001; Mearns et al., 1987; Smith et al., 1995), and would provide more insight on feeding niches occupied by various species in pond ecosystems. Therefore, the aim of this study was to quantify the feeding and swimming behaviour and the social interactions between rohu and CC, using fish tanks as a model for our polyculture ponds. We stocked the tanks with rohu (3 fish m^{-2}) and with various densities of CC (0, 1 and 2 CC m^{-2}) under fed and unfed conditions.

MATERIALS AND METHODS

Study site and tank preparation

The experiment was conducted between November 2003 and January 2004 in six tanks at the Fisheries Faculty Field Laboratory, Bangladesh Agricultural University, Mymensingh. Rectangular (2.5×0.4 m) tanks of 0.9 m height were used. In each tank, the short sides were constructed from concrete and the long sides were from glass, allowing direct observation of fish at any place in the tank. In the tanks, pond conditions were simulated: the bottom received pond sediment and the tanks were filled with pond water. The heights of the sediment and water column in each tank were 10 and 70 cm respectively. If the turbidity in the tank became too high, preventing observations in any part within the tank, the water in the tank was diluted with less turbid pond water. All tanks were treated with agricultural lime ($CaCO_3$) at a rate of 250 kg ha^{-1} ((25 g tank^{-1}), decomposed cow manure at 1,250 kg ha^{-1} (125 g tank^{-1}), urea at 31 kg ha^{-1} (3.1 g tank^{-1}) and triple super phosphate (TSP) at 16 kg ha^{-1} (1.6 g tank^{-1}) one week before fish stocking. The glass walls were covered by bamboo mats to prevent sunlight penetration other than through the surface area, like in a normal earthen pond. These bamboo mats were only removed during recording of fish behaviour.

Experimental design, fish stocking and management

The study was set up as a 3×2 factorial experiment, the factors being CC stocking density (3 levels: 0, 1 and 2 fish tank^{-1}) and feed addition (2 levels: with and without artificial feed). All treatments were executed in triplicate. All tanks were stocked with 3 rohu tank^{-1}. The range of average weights of rohu and CC stocked in the tanks were 66.5-68.3 g and 79.9-82.0 g, respectively. Fish were always stocked between 19:00 and 20:00 h. A 30% crude protein formulated diet, containing fish meal (protein: 57.5%, inclusion in feed 37%) rice bran (14%, 47%), mustard oil cake (14%, 15%) and vitamin premix (0%, 1%) was applied daily at 15 g kg$^{-0.8}$ day^{-1} starting at the day of stocking until the day of harvesting.

Temperature, dissolved oxygen (DO), pH, nitrate nitrogen (NO_3-N), total ammonia nitrogen (TAN), total nitrogen (TN), phosphate phosphorus (PO_4-P), and total phosphorus (TP) were determined before stocking of the fish at 18:00 h. During behaviour recording, water quality was measured at 6:00, 18:00 and the next day at 6:00 spanning a 24 hours time period. Temperature was measured with a centigrade thermometer, DO by the Winkler titration method (Stirling, 1985), pH with a pH meter (model: Jenway 3020). TAN and PO_4-P were analyzed spectrophotometrically (Milton Roy Spectronic, model 1001 plus; Stirling, 1985). NO_3-N (cadmium reduction), TP (acid persulphate method) and total Kjeldahl-N were determined according to APHA (1998).

At the end of the experiment, tanks were drained and all fish were harvested and weighed to the nearest 0.1 g. Specific growth rate (% body weight day^{-1}) was calculated using the formula,

$$SGR = [\ln WT_F - \ln WT_I] \times 100/T$$ (Hopkins, 1992)

with WT_F = average final fish weight (g), WT_I = average initial fish weight (g), and T = duration of the experiment (days). Water quality and growth data were collected to verify whether the fish tank environments behaved similar to ponds as used in previous experiments (Rahman et al., 2006; article in progress), and could therefore be regarded as simulated ponds.

Analysis of plankton, periphyton and benthic macroinvertebrates

Plankton and benthic macroinvertebrates were sampled at the beginning and end of the experiment. Periphyton biomass was sampled at the end of the experiment. For plankton collection a 5 L sample was passed through a 10-µm mesh plankton net. Each concentrated plankton sample was then transferred to a plastic bottle and diluted to 100 ml with 5% buffered formalin solution. Plankton numbers were estimated in a Sedgewick-Rafter (S-R) cell. A 1 ml sample was put in the S-R cell and left for 10 minutes 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 under a microscope. We used 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 planktonic organisms per litre of pond water, P = the number of planktonic organisms counted in ten fields, C = the volume of the plastic bottle holding the sample (100 ml), and L = the volume of the pond water sample (L).

In each tank, bottom mud samples were collected with an Ekman dredge and washed through a 250 µm mesh size sieve. Benthic macroinvertebrates remaining on the sieve were preserved in a 100-ml plastic bottle containing a 5% buffered formalin solution. Identification keys used for benthic macroinvertebrates were Brinkhurst (1971) and Pinder and Reiss (1983). Their density was calculated using the formula,

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

with N = the number of benthic organisms (numbers m^{-2}), Y = total number of benthic organisms counted, and A = area of bottom mud collected (cm^2). For periphyton estimation, four 2×2 cm^2 samples of periphyton (two from the concrete wall and two from the glass wall) were taken from each tank with a scalpel blade at two different depths (approximately 10 cm below the water surface and 10 cm above the sediment). The 4 samples were pooled and diluted to 100 ml with 5% buffered formalin in a plastic vial. The method applied for periphyton identification was similar to the method for plankton identification. Periphyton numbers were estimated using the formula,

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

where N = number of periphyton cells or units per cm^{-2} surface area, P = number of periphytic units counted in ten fields, C = volume of final concentrate of the sample (ml), S = area of scraped surface (cm^2).

The biovolumes of plankton, periphyton and benthic macroinvertebrates were calculated based on length and shape measurements according to Rahman et al. (2006).

Video recording and analysis

After stocking the fish, an acclimatization period of one week was included before starting behavioural observations through video recording. Two analogue video cameras (model HEL30K1A000) connected with a Quard (model NB2010S), a video cassette recorder (SANYO, model TLS-9924P) and a TV (SONY, model KV-TG21M80) were used for the recording (Figure 1). The combined camera images covered the whole tank water volume. Fish behaviour was monitored during a full 24 hour period, starting at 08:00 h with a 15 minute recording, which was repeated every 3 hours (8:00, 11:00, 14:00, 17:00, 20:00, 23:00, 2:00 and 5:00 h). After each 24 hours recording, fishes were harvested and the tanks were prepared for the next observation, starting again after a one-week acclimatization period. Treatments were assigned randomly to the tanks, and performed consecutively to keep the total observation period as short as possible.

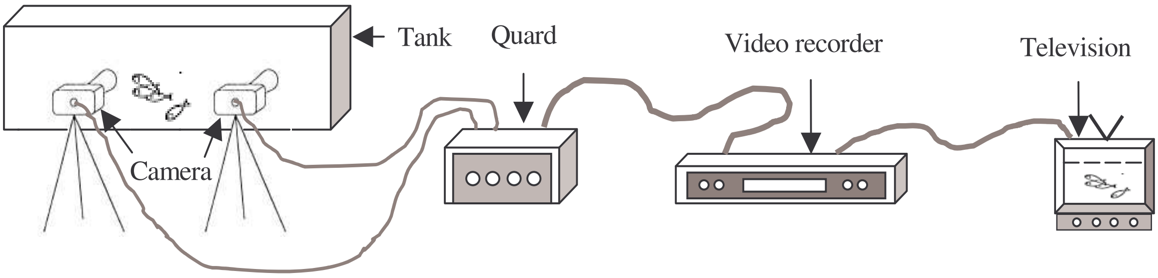

Figure 1. Video recording unit used in the recording of fish behaviour.

All video recordings per tank were analyzed for individual fish behaviour by direct observation using "The Observer®", version 4.1 software (Noldus Information Technology, Wageningen, Netherlands). All behaviour were measured on the basis of total time engaged in every 15 minutes period and expressed as percentage of total time. Types of fish behaviour included in the study related to grazing, swimming, resting and social interactions. A more detailed description of these behaviour is given in Table 1.

Table 1. Description of the behavioural variables.

Behavioural element	Variable
Grazing	Grazing in the water column
	Grazing on the tank wall
	Grazing on the bottom
Swimming	Swimming in the water column
	Swimming near the bottom
Resting	Resting
Social behaviour	All fish were scattered more than 10 cm apart (Not_t)
	Three rohu less than 10 cm apart (RRR)
	Two rohu less than 10 cm apart, all other fishes scattered (RR)
	Two or three rohu and one or two CC less than 10 cm apart (RRCC)
	One rohu with one or two CC less than 10 cm apart (RC)

If RRCC and RC occurred at the same time RRCC was prioritized, if RC and RR occurred at the same time RC was prioritized.

Data analysis

All behavioural percent data were arcsine-transformed before analysis. Water quality and food availability data were only transformed in case the data were not normally distributed. All data were analyzed through 3×2 factorial ANOVA (Gomez and Gomez, 1984) using the computer statistical package SAS (version 9.1, SAS Institute Inc., Cary, NC, USA). Factors were CC density (3 levels) and artificial feed (2 levels). If a factor or interaction was significant, differences between the means were analyzed by Tukey test for unplanned multiple comparisons of means ($P<0.05$ level of significance).

RESULTS

Grazing behaviour

Rohu grazed at least three times as long in the water column as on the tank walls, or near the bottom in all treatments (Table 2). However, its grazing behaviour was still greatly influenced by the addition of CC and artificial feed (Table 2). It decreased grazing in the water column with increasing CC density, while increasing time spent grazing on/near the bottom. Rohu also grazed more on the tank wall in the presence of CC, but more so in the presence of 1 than in the presence of 2 CC tank^{-1}. Almost a similar shift in behaviour was observed when considering the effect of artificial feed addition. Rohu decreased its time spent on grazing in the water column and increased grazing time on the tank wall and bottom when feed was administered.

CC mostly grazed on/near the bottom (at least twice as much time as in the water column) and hardly on the tank wall in all treatments (Table 3). Like in rohu, it's grazing behaviour was also influenced by it's density and by the addition of artificial feed. CC spent less time grazing on the bottom at 2 than at 1 CC tank^{-1} (Table 3). Time spent by CC on grazing in the water column and tank wall was not affected by CC density. CC decreased the time spent on grazing in the water column and on the bottom when artificial feed was applied. Feed had no effect on grazing on the tank wall by CC.

Table 2. Effects of CC and artificial feed addition on the grazing and swimming behaviour of rohu.

Variable	Significance of effects (*P* value)			Means (Tukey test)				
				CC			Feed	
	CC	Feed	CC×Feed	0	1	2	With	Without
Grazing in the water column	***	**	NS	26.6^{a}	20.7^{b}	18.3^{c}	20.8^{b}	22.9^{a}
Grazing on wall	***	**	*	3.7^{c}	7.0^{a}	5.3^{b}	6.1^{a}	4.6^{b}
Grazing on/near bottom	***	**	NS	0.9^{c}	4.1^{b}	4.4^{a}	3.4^{a}	2.8^{b}
Swimming in the water column	*	NS	NS	49.0^{b}	48.1^{b}	51.3^{a}	49.1	49.8
Swimming near bottom	***	NS	NS	3.6^{c}	11.5^{b}	13.8^{a}	10.1	9.3
Resting	***	NS	NS	17.5^{a}	8.5^{b}	6.7^{c}	11.4	10.5

CC = common carp density; Feed = artificial feed addition; CC×Feed = interaction of common carp density and feed; 0 = tanks without common carp; 1 = tanks with 1 common carp; 2 = tanks with 2 common carp; With = treatments with feed; Without = treatments without feed. Values are the percent duration of time. Mean values in the same row with no superscript in common are significantly different, according. If the effects were significant, ANOVA was followed by a Tukey test for the unplanned comparison of means (*P<0.05; **P<0.01; ***P<0.001; NS, non-significant).

Table 3. Effects of CC and artificial feed addition on the grazing and swimming behaviour of rohu.

Variable	Significance of effects (P value)			Means (Tukey test)			
				CC		Feed	
	CC	Feed	CC×Feed	1	2	With	Without
Grazing in the water column	NS	**	NS	7.3	7.1	6.3^{b}	8.0^{a}
Grazing on wall	NS	NS	NS	1.6	1.8	1.7	1.8
Grazing on bottom	***	***	*	19.1^{a}	13.9^{b}	13.1^{b}	18.3^{a}
Swimming in the water column	***	NS	NS	38.7^{b}	51.6^{a}	48.2	46.4
Swimming near bottom	***	***	NS	33.3^{a}	25.4^{b}	30.6^{a}	25.5^{b}

CC = common carp density; Feed = artificial feed addition; CC×Feed = interaction of common carp density and feed; 1 = tanks with 1 common carp; 2 = tanks with 2 common carp; With = treatments with feed; Without = treatments without feed. Values are the percent duration of time. Mean values in the same row with no superscript in common are significantly different, according. If the effects were significant, ANOVA was followed by a Tukey test for the unplanned comparison of means (*P<0.05; **P<0.01; ***P<0.001; NS, non-significant).

There was a significant interaction between CC density and feed addition on the time spent by rohu grazing on the tank walls, while for CC there was a significant interaction for grazing time on the bottom. Rohu spent more time grazing on tank walls when artificial feed was administered and this effect was higher in the presence of CC (Figure 2-A), while CC spent less time grazing on the bottom when artificial feed was available and this effects was higher in presence of 1 than in the presence of 2 CC tank^{-1} (Figure 2-B).

Swimming behaviour

Rohu mostly swam in the water column (at least 3.5 times as much as near the bottom in all treatments (Table 2)). In the presence of 2 CC tank^{-1}, the time spent for swimming in the water column was even slightly longer than in the absence or at the lower density of CC. It spent more than three times as much time swimming near the bottom in presence than in the absence of CC. The addition of feed had no effect on rohu's swimming behaviour. CC significantly decreased the resting time of rohu with increasing CC's density, whereas there was no significant effect of artificial feed on resting time of rohu.

CC also spent more time swimming in the water column than near the bottom, but never more than twice as much (Table 3). CC density had significant effects on its swimming behaviour, with more time spent swimming in the water column and less time swimming near the bottom at the higher CC density. Administration of feed caused CC to spend more time swimming near the bottom, while time spent by CC swimming in the water column was not affected by artificial feeding. There were no significant interactions between CC density and feed addition for all types of swimming activity of rohu and CC. CC was very active, not taking any rest during the observation time, hence resting behaviour could not be analyzed.

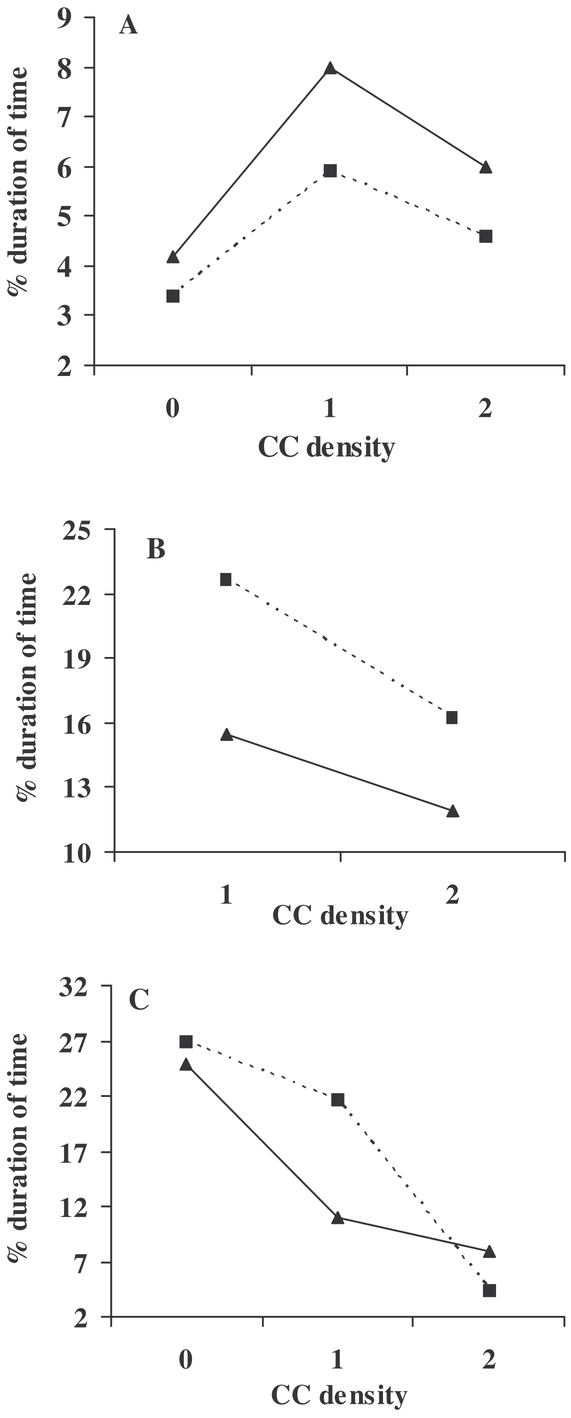

Figure 2. Interacting effects of CC density and artificial feed addition on rohu's grazing on the tank wall (A), on CC's grazing on the tank bottom (B) and rohu's inter-species schooling (RRR) behaviour (C). Dotted and solid lines indicate treatment without and with feed, respectively.

Social behaviour

Addition of CC had significant effects on all social behaviour whereas addition of artificial feed had no significant effect on any social behaviour of rohu and CC (Table 4). Rohu showed highest scattering (Not_t) and intra-species schooling (RRR and RR) behaviour in tanks without CC, followed by tanks with 1 and 2 CC, respectively. Inter-species schooling behaviour among at least two rohu and one or two CC (RRCC) also increased with CC density. The opposite was observed for inter-species schooling behaviour between/among one rohu and one or two CC (RC). RC was higher in the tanks with 1 CC than in the tanks with 2 CC. We only found a significant interaction between CC density and artificial feed addition on the intra-species schooling of all three rohu (RRR). RRR was lower with increasing CC density, and there was no overall effect of feed addition, but in the treatments with 1 CC feed addition decreased RRR further than in the absence of artificial feed (Figure 2-C). No intra- or inter-species aggressive behaviour was observed during the observation period.

Table 4. Effects of common carp and artificial feed on the social behaviour of common carp and rohu.

Variable	Significance of effects (*P* value)			Means (Tukey test)		
	CC	Feed	CC×Feed	0	1	2
Not_t (%)	**	NS	NS	40.1^{a}	20.1^{b}	6.0^{c}
RRR (%)	**	NS	*	25.8^{a}	16.4^{b}	6.2^{c}
RR (%)	**	NS	NS	34.1^{a}	16.4^{b}	11.1^{c}
RC (%)	*	NS	NS	-	25.6^{a}	15.3^{b}
RRCC (%)	**	NS	NS	-	21.4^{b}	36.2^{a}

CC = common carp density; Feed = artificial feed addition; CC×Feed = interaction of common carp density and feed; 0 = tanks without common carp; 1 = tanks with 1 common carp; 2 = tanks with 2 common carp. Values are the percent duration of time. Mean values in the same row with no superscript in common are significantly different. If the effects were significant, ANOVA was followed by a Tukey test for the unplanned comparison of means (*$P<0.05$; **$P<0.01$; NS, non-significant).

Water quality, natural food availability and fish growth

In the current study, the effects of CC and artificial feed on water quality, natural food availability and fish growth had almost similar effects as in our pond study (Table 5). The levels of the values in the pond and tank experiments were sometimes different, but when ordering the values from low to high the same order was found in pond and tank experiments in most cases. Even if the order was not the same (e.g., the PO_4-P concentration was highest in the treatment with the lower CC density in the pond experiment, while it was highest in the treatment with the higher CC density in the tank experiment), it always involved relatively small differences that were statistically non-significant. This means that we did not find any contradictory results between the pond and tank measurements.

DISCUSSION

Our study is the first study that quantified the swimming and grazing behaviour of rohu in a tank. A similar type of experiment was conducted by Rueda et al. (2004) with African catfish *Clarias gariepinus* in aquaria supplied with only artificial feed. They found that around 75% of the time fishes were swimming while only 2% was spent eating in 24 hours time. In our study, rohu spent more than half of its total time swimming and around one-third of its total time grazing. This longer grazing time could be explained by the dependency of rohu on natural food (Rahman et al., 2006), which takes longer to be collected by the fish. Rohu spent the majority of its time swimming (≈50%) and grazing (≈25%). When grazing, rohu grazed the water column in 65-85% of its time, and swam 79-93% of its swimming time in the water column. This indicates that rohu lives and feeds mainly in the water column, which is in concordance with Dewan et al. (1991), Wahab et al. (1994) and Azim et al. (2004), who considered rohu as a water column feeder. CC spent 63-68% of its grazing time on the bottom and in our pond studies we also showed around 75% of the CC's diet consisted of benthic macroinvertebrates. CC spent 39-52% of its total time near the bottom and the remaining time in the water column. On the basis of these above results CC can be considered as a bottom feeder, mainly feeding on benthic macroinvertebrates, but living both in the water column and near the bottom. This result is in agreement with the commonly accepted statement that CC is a bottom feeder (Zambrano and Hinojosa, 1999; Zambrano et al., 2001; Parkos III et al., 2003).

Table 5. Effects of common carp and artificial feed on different water quality, natural food availability and fish growth parameters based on two-way ANOVA in the pond and tank experiments.

Variable	System	Means (Tukey test)				
		Common carp density			Feed addition	
		0	Low	High	With	Without
DO (mg L^{-1})	Tank	6.2^{a}	5.9^{ab}	5.5^{b}	5.6^{b}	6.1^{a}
	Pond	7.1^{a}	6.4^{b}	6.1^{c}	6.3^{b}	6.7^{a}
pH range	Tank	6.6-9.2	6.7-9.3	6.5-8.4	6.7-9.3	6.5-8.9
	Pond	7.2-8.4	6.5-8.3	6.5-8.2	6.5-8.3	6.8-8.4
NO_3-N (mg L^{-1})	Tank	0.38	0.43	0.43	0.55^{a}	0.28^{b}
	Pond	0.78^{b}	0.96^{a}	0.95^{a}	0.96^{a}	0.84^{b}
TAN (mg L^{-1})	Tank	0.13	0.15	0.16	0.18^{a}	0.10^{b}
	Pond	0.25^{b}	0.32^{a}	0.31^{a}	0.33^{a}	0.26^{b}
TN (mg L^{-1})	Tank	0.73^{b}	**0.78^{ab}**	**0.83^{a}**	0.98^{a}	0.59^{b}
	Pond	1.52^{b}	**1.93^{a}**	**1.81^{a}**	1.88^{a}	1.62^{b}
PO_4-P (mg L^{-1})	Tank	0.23^{b}	**0.27^{a}**	**0.28^{a}**	0.31^{a}	0.21^{b}
	Pond	0.74^{c}	**1.07^{a}**	**1.00^{b}**	1.01^{a}	0.87^{b}
TP (mg L^{-1})	Tank	0.42^{b}	**0.48^{a}**	**0.49^{a}**	0.58^{a}	0.36^{b}
	Pond	1.28^{c}	**1.67^{a}**	**1.58^{b}**	1.57^{a}	1.44^{b}
Total phytoplankton (mm^{3} L^{-1})	Tank	0.06^{b}	0.14^{a}	0.13^{a}	0.13^{a}	0.10^{b}
	Pond	0.27^{b}	0.35^{a}	0.29^{b}	0.31	0.29
Total zooplankton (mm^{3} L^{1})	Tank	0.48^{c}	1.56^{a}	1.08^{b}	1.41^{a}	0.67^{b}
	Pond	0.04^{c}	0.07^{a}	0.06^{b}	0.06^{a}	0.05^{b}
SGR of rohu	Tank	**0.9**	1.2	**1.1**	1.2	1.0
	Pond	**1.39^{b}**	1.55^{a}	**1.35^{b}**	1.51^{a}	1.35^{b}
SGR of common carp	Tank	-	1.3^{a}	1.0^{b}	1.3	1.0
	Pond	-	1.59^{a}	1.38^{b}	1.75^{a}	1.22^{b}

0 = treatments without common carp; Low = treatments with 0.5 (pond) or 1 (tank) common carp m^{-2}; High = treatments with 1 (pond) or 2 (tank) common carp m^{-2}; With = treatments with feed; Without = treatments with no feed; SGR = specific growth rate (% body weight day^{-1}). If the effects were significant, ANOVA was followed by a Tukey for the unplanned comparison of means. Mean values in the same row with no superscript in common differ significantly. Values that are marked bold indicate a difference in the order of values between tank and pond systems. Note however, that this always involves non-significant differences.

Association with other species may influence feeding and swimming behaviour. For example, when three species of sunfish, the bluegill (*Lepomis macrochirus*), green sunfish (*L. cyanellus*) and the pumpkinseed (*L. gibbosus*) are stocked separately in ponds, each species prefers to forage mostly on invertebrates in the vegetation, but when they are stocked together, the diet of the green sunfish showed no change, the bluegill concentrated partially on prey from the water column, especially zooplankton, and the pumpkinseed largely fed on prey from the sediment (Werner and Hall, 1976; 1977; 1979). In our study, CC was very active, not taking any rest during the observation time, while rohu increased its activity and reduced resting with 51-62% in the presence of CC. Rohu also reduced intra-species schooling and scattering time, while increasing time together with CC (RC+RRCC, Table 4) up to 47-51% of its total time with increasing CC density. This is probably caused simply by the increased probability of rohu being together with CC as a result of the higher number of CC being present. Moreover, CC influenced rohu to shift from resting and grazing in the water column to grazing and swimming near the tank bottom (Table 2).

Rohu mainly grazed on natural food and preferred zooplankton above phytoplankton (Rahman et al., 2006). This food preference might be associated with the grazing behaviour of rohu. Artificial feed had fertilizing effects that increased zooplankton density. Rohu grazed more time during the day than night (article in progress), and during the day most of the zooplankton stays on or near the bottom (DeStasio, 1993; Jeppesen, 1998; Burks et al., 2002). This resulted rohu might have grazed higher time near the bottom in the tanks with artificial feed than in the tanks without artificial feed. Rohu's zooplankton preference also influenced grazing on periphyton from the tank wall. In the tanks periphyton biomass was mainly composed of zooplankton and almost 9 times higher than phytoplankton biomass. The time spent on grazing on the tank wall by rohu was strongly positively correlated ($r = 0.87$, $P<0.05$) with periphyton biovolume, which apparently resulted in rohu spending more time in grazing on the tank wall when the periphyton biomass was high. These findings are in concordance with Wahab et al. (1999), Ramesh et al. (1999) and Azim et al. (2001), who mentioned that rohu is a very active periphyton grazer.

It is clear that rohu mainly benefited from ingesting zooplankton by grazing on the tank wall and near the tank bottom. Rohu's overall total grazing time (tank wall plus tank bottom) was highest in tanks with 1 CC (1.4 times higher than in tanks without CC), followed by tanks with

2 (1.1 times higher than in tanks without CC) and 0 CC. As a result, rohu's overall ingestion of zooplankton in the different treatments might have the same ranking. Similarly, the growth performance of rohu was relatively higher in tanks with low density of CC (specific growth rate: 1.2% body weight day^{-1}), followed by high density (1.1) and no CC (0.9). These finding indicate that the sum of grazing on tank wall and tank bottom is a good indicator of the growth performance of rohu. This relationship holds both in our fed and non-fed tanks.

The possible mechanism by which CC behaviour influenced its growth performance might be related to its stocking density. At lower densities CC stays longer near the bottom, while at higher densities it spends more time in the water column. This might simply be an effect of the higher fish density, resulting in more interactions between fish on the bottom and a higher tendency of fish to swim in the water column. At lower densities CC was more active in grazing on the bottom and showed a higher growth rate (specific growth rate: 1.3% body weight day^{-1} at low density compared to 1% day^{-1} at high density). This finding suggests that grazing time on the tank bottom is a good indicator of CC growth performance in absence of artificial feed. However, CC prefers artificial feed above natural food (Rahman et al., 2006). Generally, CC take less time for grazing if feed/food is readily available, as in the case of artificial feed addition. The reason for this is that fish can collect artificial feed within a shorter period. This is supported by our results in which we can see that the time spent grazing is less in fed than in non-fed tanks.

There were differences between in water quality, natural food and fish growth variables between pond and tank experiments. Especially the levels of the water quality parameters and natural food biovolumes were generally lower in the tank experiments (Table 5), even the higher density of CC in the tanks was even lower than the lower density in the ponds. The reasons for this were most probably: (1) water dilution with pond water when the turbidity became too high to enable observations of fish behaviour and (2) short culture period: the culture period of the present study was very short (7 days) compared to the pond study (4.5 months). However, the addition of CC and artificial feed did have similar trends in the effects on water quality, natural food availability and fish growth in our tank and pond studies. The orders of the values were never significantly contradictory. This strongly suggests that we can regard our tanks as good models for our ponds and that the observed behaviour of common carp and rohu in the tank experiment gives a good indication of behaviour in ponds.

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Chapter 6

General Discussion

In semi-intensive aquaculture, the flow of nutrients through the food web depends to a large extent on nutrient availability in the water column. Diffusion is the main mechanism by which nutrients become available from the sediment into the water column. Thus, these nutrient fluxes across the sediment-water interface enhance production (Wetzel, 1975; Avnimelech and Kochva, 1999). In aquaculture ponds, the fluxes of nutrients between sediment and water column are enhanced by fish-driven resuspension, which quickly becomes more important than diffusion (Ritvo et al., 2004; Tarvainen et al., 2005). Fish-driven resuspension is most important in low-input ponds, where fish production fully depends on natural food availability. One objective of this thesis was to measure the effects of common carp, which stirs up sediments (Sidorkewicj et al., 1998; Zambrano et al., 2001), on nutrient concentrations, nutrient accumulation, natural food availability and total fish production in polyculture. Another goal was to analyse the underlying mechanisms explaining the synergism in growth and production between rohu and common carp in semi-intensive polyculture ponds. If stocking of common carp leads to better nutrient retention in combination with increased production, it can be used to improve farm productivity and sustainability.

We first studied the relationships among limnological, food intake and fish growth related parameters using multivariate techniques. Secondly, we studied the effects of artificial feed addition and common carp density on natural food availability, food preference and growth in rohu ponds. Thirdly, the effects of these factors on nutrient concentrations in the water column, overall water quality and the percentage of input nutrients retained in different types of natural food were studied. Finally, linkages between changes in behaviour and production, which could (partially) explain the observed synergism between rohu and common carp were analysed.

In this Chapter the results from the above-mentioned studies are synthesized and cross-checked, while summarizing the major conclusions. We also review the used approach and identify areas for further research.

Common carp affected nutrient concentrations in the water column

Three different densities (0, 0.5 and 1 individual m^{-2}) of common carp were added to ponds stocked with 3 rohu m^{-2}. Water quality parameters changed with addition of common carp

(Chapter 2 and 4), although in all treatments water quality remained favourable for fish growth. The highest total nitrogen and total phosphorus concentrations were observed in treatments with 0.5 common carp m^{-2}, followed by treatments with 1 and 0 common carp m^{-2}. The positive effects of stocking 0.5 common carp m^{-2} on nitrogen and especially phosphorus resuspension were partially undone at the higher density of 1 common carp m^{-2}. This effect was more pronounced when artificial feed was given (Chapter 2 and 4). We concluded that the key factors controlling nutrient concentrations in the water column included (i) common carp presence, (ii) common carp density, and (iii) nutrient availability in the sediment. Among these key factors, common carp presence always enhanced nutrients availability in the water column, irrespective of artificial feeding or common carp density. Artificial feeding only enhanced the effect of common carp presence. Increased nutrient availability in the water column due to common carp presence has been well documented (Yashouv, 1971; Milstein et al., 1988; Milstein et al., 2002) but the mechanisms explaining the cross linkage between the key factors have been insufficiently explored.

Not all shallow aquatic ecosystems will react similarly to common carp density. Parkos III et al. (2003) observed higher total phosphorus concentrations with increasing common carp biomass in non-fertilized and non-fed enclosures in shallow water bodies. In our study, the opposite was observed. Possible explanations include (i) differences in the P solubility due to differences in pH and alkalinity, (ii) differences in trophic state due to differences in type and amount of nutrient input and (iii) differences in sediment properties and the magnitude of sediment-water column interactions.

Common carp density affected plankton and benthic macroinvertebrate availability

The major natural food types in ponds are phytoplankton, zooplankton, benthic macroinvertebrates and detritus. The amounts of these natural foods in lakes and ponds (Table 1) are influenced by management factors, such as fish species combinations in polyculture, fish stocking density and ratio, and nutrient input quality and quantity (Milstein, 1993; Diana et al., 1997). Fish feeding habits have an important influence on natural food quantity, both directly by consumption and indirectly through influencing the food web and nutrient availability. For instance, bottom feeders, such as common carp, searching for benthic macroinvertebrates resuspend sediments, thereby influencing nutrient availability in the water column, which in turn affects photosynthesis and subsequently phytoplankton production.

Table 1. Reported biomass of natural foods in fish ponds.

Food types	Biomass	System	References
Phytoplankton	44-65 (g FM m^{-3})	Pig-fish integrated pond	Yang, 1994.
Phytoplankton	29-36	Integrated fish pond	Takamura et al., 1995.
Phytoplankton	1-71 (g FM m^{-3})	Shrimp pond	McIntosh et al., 2001.
Phytoplankton	121	Fish pond	Tang, 1970.
Phytoplankton	265-352 (mm^3 FM m^{-3})	Fish pond	Present study (Chapter 3).
Periphyton	7-25	Fish pond	Azim et al., 2001.
Periphyton	24-145	Fish pond	Azim et al., 2002b.
Periphyton	7-18	Concrete tank	Keshavanath et al., 2002.
Zooplankton	3-5	Integrated fish pond	Takamura et al., 1995.
Zooplankton	4-10 (g DM m^{-3})	Nursery pond	Molodtsova-Zaikina, 1977.
Zooplankton	10-13 (g FM m^{-3})	Pig-fish integrated pond	Yang, 1994.
Zooplankton	10	Fish pond	Tang, 1970.
Zooplankton	2	Eutrophic pond	Iwakuma et al., 1989.
Zooplankton	42-67 (mm^3 FM m^{-3})	Fish pond	Present study (Chapter 3).
Benthos	9-36	Nusery pond	Molodtsova-Zaikina, 1977.
Benthos	15-70	Pond	Wade and Stirling, 1999.
Macroinvertibrate	4	Pond	Oerlti, 1993.
Macroinvertibrate	3.2-6.2 (cm^3 FM m^{-2})	Fish pond	Present study (Chapter 3).
Detritus	130	Pig-fish integrated pond	Yang, 1994.
Detritus	83	Pig-fish integrated pond	Yang, 1994.

All biomass expressed as g dry matter per m^2 except where indicated differently (FM = fresh matter; DM = dry matter).

In this study, common carp presence increased phytoplankton biomass by 8-33% and zooplankton biomass by 36-60% (Chapter 3). Common carp affected phytoplankton and zooplankton biomass directly by grazing and indirectly by nutrient resuspension. The indirect effect was more pronounced than the direct effect (Chapter 2), indicating that nutrient levels in the water column to a large extent control phytoplankton and zooplankton biomass. However, the positive effects of stocking 0.5 common carp m^{-2} were partially lost when stocking 1 common carp m^{-2}. Possible explanations include:

1. *Lower availability of phosphorus*: At the higher density of common carp (1 m^{-2}), more sediment was resuspended including phosphorus, which might form inorganic particles e.g., with iron as iron (III) phosphate (Holdren and Armstrong, 1980; Boyd, 1995). Hence phosphorus availability was reduced, resulting in the observed lower phytoplankton biomass in ponds with 1 common carp compared to 0.5 common carp m^{-2} (Chapter 2). Phytoplankton availability in turn influenced zooplankton availability.
2. *Light limitation*: A higher common carp density resulted in more sediment particles in the water column, reducing light penetration and photosynthesis, which in turn reduced phytoplankton and zooplankton production (Roberts et al., 1995; Parkos III et al., 2003).
3. *Higher grazing pressure*: A higher common carp density resulted in a higher grazing pressure on plankton.

In addition, stocking 0.5 and 1 common carp m^{-2} reduced benthic macroinvertebrate density by 40 and 48%, respectively (Chapter 3). This suggests that common carp grazing directly affected the benthic macroinvertebrate biomass (Riera et al., 1991; Tatrai et al., 1994; Zambrano and Hinojosa, 1999).

Common carp density influenced rohu behaviour

Direct observations of rohu and common carp confirmed that rohu is a water column feeder, mainly feeding on plankton, while common carp is a bottom feeder, mainly feeding on benthic macroinvertebrates (Chapter 5). In presence of common carp, rohu's grazing, swimming and social behaviour changed: (i) active time increased by 51-62%, (ii) intra-species schooling time decreased by 55-71% and (iii) 47-52% of the time was spent in the company of common carp. The net result was that rohu spent relatively more time grazing near the bottom instead of in the water column where the density of zooplankton is smaller. Hence, in presence of common carp, rohu ate more zooplankton, which resulted in better growth (Figure 1). In general, rohu liked to be in the presence of common carp while common carp did not mind.

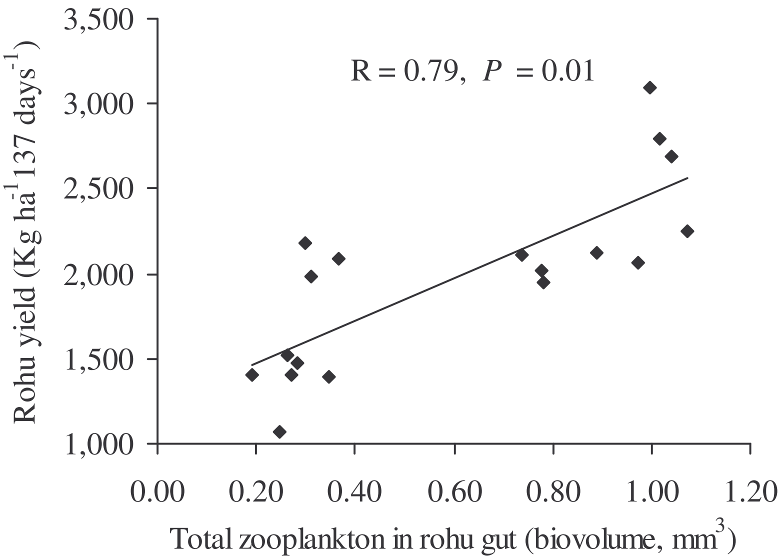

Figure 1. Relationship between rohu's zooplankton ingestion and rohu yield obtained from different ponds.

Behaviour of common carp also changes with density. At the highest density, common carp spent relatively more time swimming in the water column, at the expense of time grazing near the bottom. This in part explains the reduction in individual growth at high common carp stocking densities, but more research is needed to understand how stocking density triggers the observed changes.

Common carp addition increased pond production

Fish production in carp polyculture ponds in south Asia varies between 1,200 and 10,100 kg ha^{-1} yr^{-1} (Table 2). Productions of 11,800 kg ha^{-1} yr^{-1} with 0.5 common carp and artificial feeding and of 4,900 kg ha^{-1} yr^{-1} in carp free non-fed ponds obtained in our experiments, clearly fall in the higher production ranges. Specific growth rates of rohu and common carp were higher in treatments with 0.5 common carp m^{-2} (rohu: 1.55 % body weight day^{-1}, common carp: 1.59) than with 1 common carp m^{-2} (rohu: 1.35, common carp: 1.38), whereas growth performance of rohu was more or less similar in treatments with 0 (1.39) and 1 common carp m^{-2}. In the presence of 0.5 common carp m^{-2} rohu production increased 1.4

times and total production 2 times, compared to common carp-free ponds. In the presence of 1 common carp m^{-2} total pond production increased 1.7 times, because of increased common carp production, while rohu production was not affected compared to common carp-free ponds.

Table 2. Reported yield in different polyculture systems.

Nutrient source	Species	Yield (Kg ha^{-1} yr^{-1})*	References
	Indigenous and exotic carp	7,634	Chaudhuri et al., 1978.
	Indigenous and exotic carp	10,183	Mathew et al., 1988.
Feed	Indigenous and exotic carp	2,836-3,062	Hossain et al., 1994.
Feed	Rohu, catla, common carp and silver carp	5,542	Wahab et al., 1995.
Feed	Rohu, catla, mrigal, common carp and silver carp	6,768	Wahab et al., 1995.
Feed	Rohu, catla, mrigal and silver carp	3,909	Wahab et al., 1995.
	Indigenous and exotic carp	3,120-4,068	Miah et al., 1997.
	Indigenous and exotic carp	2,829	Salimullah et al., 1998.
Feed	Indigenous and exotic carp	3,604-5,508	Salimullah et al., 1998.
	Rohu, and kalibaus	1,217	Wahab et al., 1999.
Periphyton	Rohu, and kalibaus	2,178	Wahab et al., 1999.
Feed	Indigenous and exotic carp	3,404-5,446	Kanak et al., 1999.
Periphyton	Rohu and common carp	3,389	Ramesh et al., 1999.
	Rohu and catla	2,340	Azim et al., 2002a.
Periphyton	Rohu, catla and kalibaus	9,344	Azim et al., 2002a.
Feed	Rohu, catla and kalibaus	6,205	Azim et al., 2002b.
Periphyton	Rohu, catla and kalibaus	6,619	Azim et al., 2002b.
	Rohu, catla and kalibaus	4,202	Azim et al., 2002b.
	Rohu and common carp	5,944-7,015	Present study (Chap. 3)
Feed	Rohu and common carp	10,201-11,808	Present study (Chap. 3)

*Reported values were extrapolated to annual production. Seasonal differences in growth and production were not considered.

All ponds were fertilized. Some ponds received artificial feed (referred to as "Feed"), some ponds received substrate for periphyton development (referred to as "Periphyton"). Indigenous carp mainly includes rohu *Labeo rohita*, catla *Catla catla*, mrigal *Cirrhinus cirrhosus* and kalibaus *Labeo calbasu*. Exotic carp mainly includes silver carp *Hypophthalmichthys molitrix*, bighead carp *Aristichthys nobilis*, black carp *Mylopharyngodon piceus*, common carp *Cyprinus carpio*, and grass carp *Ctenopharyngodon idella*.

Common carp density influenced nutrient accumulation

The nitrogen and phosphorus retention efficiency in fish biomass almost doubled in presence of common carp, which caused more nutrients to pass through the pond's food web, while fewer nutrients accumulated in the sediment. In presence of common carp, nitrogen and phosphorus accumulation in the sediment were reduced by 17-22% and 34-36%, respectively. A higher percentage of the input nutrients ended up in plankton and detritus in treatments with 0.5 common carp m^{-2}, compared to treatments with 1 common carp m^{-2} (Chapter 4). The opposite was observed in the sediment. Common carp density above 0.5 common carp m^{-2} mainly affected the phosphorus (PO_4-P) availability for primary and subsequently secondary production. Hence, increasing the stocking density above 0.5 common carp m^{-2} had a negative effect on fish production and plankton availability (Chapter 3 and 4). Table 3 and Figure 2 represent the effects of common carp density and artificial feed on phosphorus availability and the cascade of events through the food web.

Table 3. Effects of common carp density and artificial feed on phosphorus resuspension, availability in the water column and sediment, and natural food availability.

Effects	0.5C	1C	Feed
P resuspension	+	+ +	NE
P precipitation	+	+ +	0
Transparency	- -	-	-
Phytoplankton	+	+	+
Zooplankton	+ +	+	+ +
Benthic macroinvertebrates	-	- -	+
P in the water column	+ +	+	+
P in the sediment	-	-	+

+ and – indicate positive and negative effects respectively. Double symbols (+ + and - -) indicate higher effects than single symbols (+ and -). 0.5C, 1C and Feed indicate the treatments with 0.5 common carp m^{-2}, treatments with 1 common carp and treatment with feed, respectively. NE = no effect, 0 = neutral.

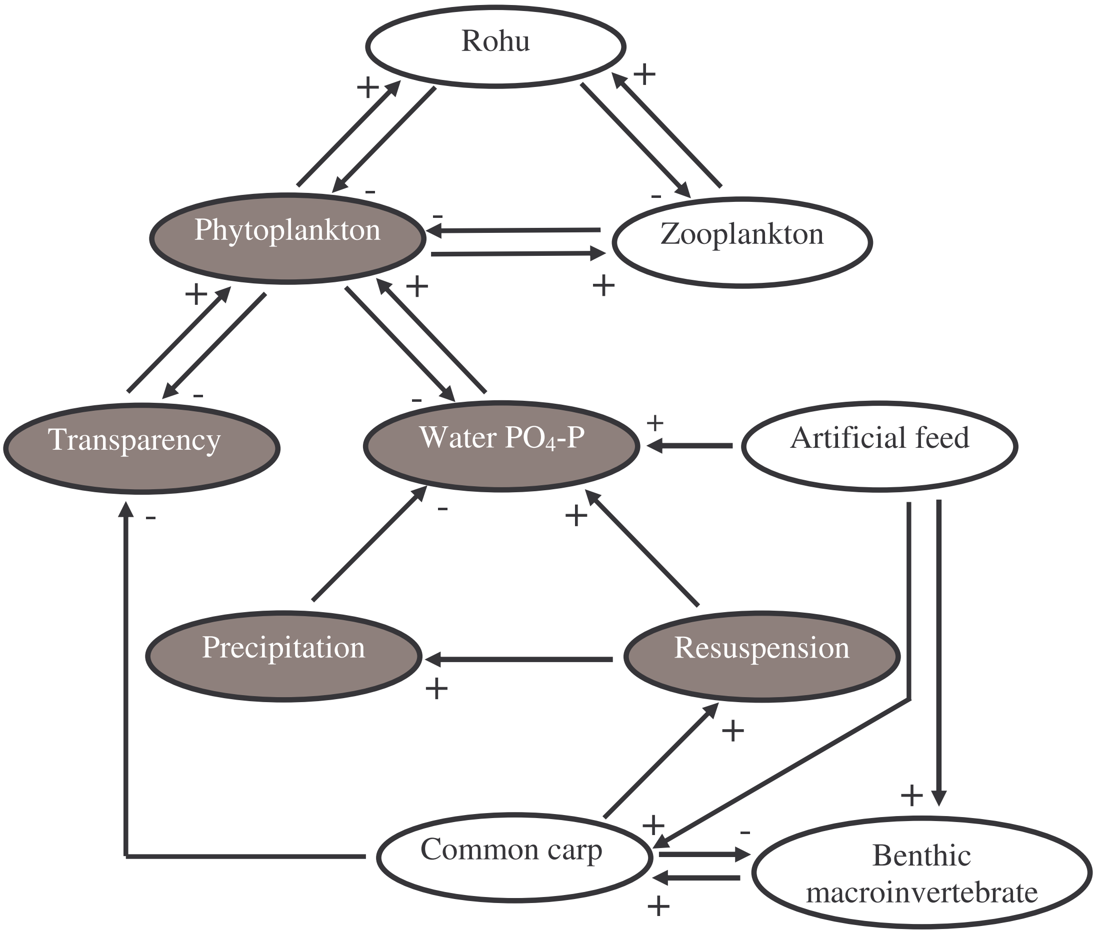

Figure 2. Schematic representation of the effects of common carp and artificial feed on PO_4-P and natural food availability and fish growth. + and – indicate positive and negative effects, respectively. Filled ellipses indicate processes and states that illustrate the influence of common carp and artificial feed on primary production.

Food ingestion vs fish growth

Rohu's electivity indices were positive for all zooplankton groups and negative for all phytoplankton groups, except Bacillariophyceae (Figure 3), confirming that rohu preferred zooplankton above phytoplankton. Rohu shifted from phytoplankton to zooplankton when zooplankton became more abundant, as a result of artificial feeding (Chapter 2). Zooplankton ingestion and rohu production were positively correlated (Figure 1), while common carp production was positively correlated with artificial feeding (Chapter 2). Common carp preferred benthos, followed by zooplankton and phytoplankton in non-fed ponds. In fed ponds, the food preference of common carp shifted from zooplankton and benthic macroinvertebrates to artificial feed (Chapter 2). This change in feeding behaviour of common carp allowed rohu to eat more zooplankton in fed ponds. We concluded that artificial feed increased zooplankton availability mainly through two ways: (i) directly by increasing availability, and (ii) indirectly by shifting feeding behaviour of common carp from zooplankton to benthic macroinvertebrates.

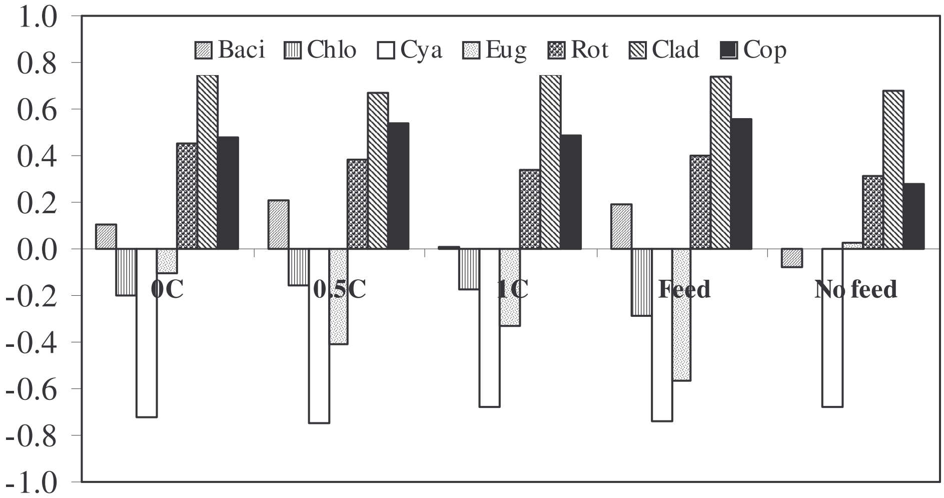

Figure 3. Electivity index of rohu in different groups of plankton in different treatments. 0C, 0.5C, 1C, Feed and No feed indicate treatments with 0, 0.5, 1, feed and no feed, respectively. Baci, Chlo, Cya, Eug, Rot, Clad and Cop indicate Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae, Rotifera, Cladocera and Copepoda, respectively. Values on Y axis indicate the electivity index (E), calculated as, E = (Pg-Pw)/(Pg+Pw) (Ivlev, 1961), where Pg is the relative content of any ingredient in the gut expressed as % of the total ration, and Pw is the relative proportion of the similar item in the pond water expressed as %. Positive values of E indicate the selection of a particular food item while a negative values indicate avoidance.

Applicability

Stocking 0.5 common carp m^{-2} in rohu ponds (3 rohu m^{-2}) had a synergistic effect on rohu and total pond production. Since the majority of the supplied nutrients to ponds accumulate in the sediment, stocking a bottom feeder like common carp will reduce nutrient losses and increase fish production. Although our findings are based on general processes that can be found in many different ponds all over the world where polyculture is applied, care should be taken to directly extrapolate results to different regions for different species combinations. Therefore, efforts to improve livelihoods of fish farming households by stocking bottom feeders should be first tested locally at pilot level before starting widespread dissemination.

Searching outside south Asia for locally available combinations of benthivorous and planktivorous species exhibiting similar synergistic effects as common carp and rohu is recommended. When promising species combinations are identified a proper testing of stocking densities and ratios is recommended. Investigating additional species with planktivorous and benthivorous is needed to further improve our understanding of food webs in fed and non-fed ponds.

Methodological limitations of this study

The natural food intake was quantified using a light microscope. Numerous food particles could not be identified, especially when partially digested. In this study, all unidentified food particles including the various ingredients mixed in the artificial feed were neglected. In addition, the numbers of plankton and benthic macroinvertebrate were converted into biovolume based on average values found in literature. Hence the accuracy of food biomass intake calculations is debatable. More detailed research is recommended, especially concerning biovolumes and nutritional value of the various natural food particles.

Behaviour observations were carried out in 1 m^{-2} (2.5×0.4 m) tanks. Although care was taken to imitate pond conditions in the tanks as good as possible, conditions were not identical. An example is the difference in space between ponds and tanks. Space availability strongly influences fish behaviour (Hossain et al., 1998; Rueda et al., 2004). Changes in space availability negatively influenced growth and stress responses (e.g., swimming and air breathing increased in African catfish) (Haylor, 1991, 1992; Rueda, 2004). Therefore, before starting the experiment, several trials were conducted with different shapes and sizes of tanks to determine the optimal tank design for observing fish behaviour. It proved not to be possible to make video observations beyond a tank width of 0.4 m, while making the glass walls of the tanks longer than 2.5 m was not possible considering the strength of glass windows locally available.

Recommendations for future studies and final remarks

There are many computer models simulating aquaculture production in ponds (Cuenco et al., 1985; Machiels and Henken, 1986; van Dam and Pauly, 1995; van Dam and de Vries, 1995; van der Meer and van Dam, 1998) but none of these models distinguishes between natural foods and artificial feed utilization. Most of the models assumed that fishes only eat the artificial feed supplied to the pond, and do not eat the natural food present. However, reality is different as most of the production in polyculture ponds is realized based on natural foods. Making the distinction between natural foods and artificial feed intake would increase the accuracy of these types of models. Moreover, all models work on a single fish species only. Pond aquaculture models with two or more species, including both artificial feed and natural food could play a significant role in understanding the complex ecology of polyculture production systems.

Also in the present study it was not possible to estimate the contribution of artificial feed and different types of natural food to fish growth. Moreover, the quality of artificial feed and different types of natural feed differ. The reported maximum gross energy content of algae, benthos and detritus are 17, 32 and 12 KJ per g dry matter, respectively, whereas for algae, zooplankton and detritus, the highest protein content are 24, 72 and 14 % (Bowen et al., 1995). When fishes consume two or more foods with different natural stable isotope ratios, the fraction that each food contributed to the diet can be calculated from the resulting stable isotope ratios in the fish (Schroeder, 1983; Fry et al., 1983; Anderson et al., 1987). Therefore, in addition to advanced simulation models, studies with labelled ^{13}C or ^{15}N ingredients can help in elucidating food webs in ponds and are highly recommended for further study.

An innovative approach of this study was to quantify natural food availability and food intake volumetrically, and not numerically as done in most other studies. As explained above, the biovolumes used in this study are debatable. The approach is however sound, and if more detailed information on the biovolume of different plankton species and other natural foods would be available, it would quickly improve our understanding of food webs in ponds. Therefore, studies on biovolumes of natural foods present in ponds are recommended (Yurkowski and Tabachek, 1979).

The major strength of this study was that it looked at the combined effects of stocking different densities of benthivorous fishes and artificial feed addition, simultaneously considering water quality, nutrients accumulation, natural food availability, feed intake and behaviour. Various previous studies considered some of these effects, but focussed on either water quality or food availability and intake (Tatrai et al., 1994; Tatrai et al., 1997; Jana and Chakrabarty, 1997; Barton et al., 2000; Lougheed and Chow-Fraser, 2001). The approach to look at these aspects in combination proved fruitful. In addition, the approach to link treatments in ponds to behavioural observations is new. Results showed that behavioural observations are equally important as water quality and feed intake monitoring to explain synergism in polyculture. By combining the results from the different studies, our understanding of changes in food web ecology as well as nutrients dynamics due to addition of a benthivorous species and feeding was improved. To further deepen our knowledge of the feeding ecology in polyculture ponds, a similar approach is recommended for studying polyculture ponds with a third species added.

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Summary

Food Web Interactions and Nutrients Dynamics in Polyculture Ponds

Artificial feed and fertilizers are the main sources of nutrients supporting fish growth in aquaculture ponds. The majority of the added nutrients are lost to the sediment, where they are no longer available for natural food production. By increasing resuspension of the sediment through the introduction of benthivorous fish, nutrient loss may be reduced, because of the re-mobilisation of nutrients from the sediment. The effects of addition of benthivorous fish and/or artificial feed in fertilized ponds have mostly been studied separately. Therefore, this thesis focuses on the integrated study of the (interacting) effects of the addition of artificial feed and a benthivorous fish species on overall nutrient dynamics, pond ecology, and growth and production of fish in polyculture ponds. To achieve this we used rohu (*Labeo rohita* Hamilton) and common carp (*Cyprinus carpio* L.), two cyprinid species, because rohu-common carp polyculture is becoming popular practice in Bangladesh. Common carp is benthivorous, stirring up bottom sediment, resulting in nutrient resuspension, while rohu is planktivorous and an efficient plankton grazer. Three common carp densities (0, 0.5 and 1 individual m^{-2}) were applied with a fixed density of rohu (3 individuals m^{-2}) in both artificially fed and unfed ponds. The aim of the study was to quantify the effects of feed and common carp addition and their interactions on water quality, nutrient accumulation, natural food production, behaviour, growth and yield of the fish. Moreover, we tried to find out whether there would be a common carp density that would be optimal for fish production in rohu ponds.

The present thesis was divided into three parts. The first part was a literature review on the status of carp polyculture and role of common carp and artificial feed on biotic and abiotic components in ponds or lakes. The second part analyzed changes of various biotic and abiotic parameters and measured synergistic effects triggered by the rohu-common carp combination. The third part monitored changes in swimming and grazing behaviour, and social interactions between rohu and common carp.

- The first part of this study (Chapter 1) focused on the positive and negative effects of benthivorous fishes on nutrients and natural food availability. Benthivorous fishes stimulated mineralization of organic matter, liberation of nutrients from sediment to the water column and primary production. The density of benthivorous fishes affected turbidity and grazing pressure on natural food, which in turn affected natural food availability. Therefore, the density of benthivorous fishes will affect the overall performance of any polyculture system.

- Part 2 includes Chapters 2, 3 and 4.

◊ Chapter 2, explored the links between water quality parameters, different types of food resources available, fish diet composition and fish growth/production. Common carp increased bioavailable nitrogen and phosphorus, a process that was enhanced by the addition of artificial feed. The effects of common carp were more pronounced in treatments with 0.5 than 1 common carp m^{-2}. PO_4-P concentration was strongly correlated with phytoplankton and zooplankton biomass. One of the major findings was that rohu growth was best explained by natural food intake, while common carp growth was best explained by artificial feed addition and negatively correlated with natural food ingestion. Results indicated that common carp benefited directly from artificial feed addition while rohu benefited indirectly from the boost in natural food availability triggered by the fertilizing effect of the artificial feed.

◊ In Chapter 3, the effects of different densities of common carp (0, 0.5 and 1 individual m^{-2}) on natural food availability, natural food ingestion uptake and fish growth were compared under fed and unfed conditions. Stocking 0.5 common carp m^{-2} resulted in the highest observed natural food availability, the highest natural food ingestion, the highest fish growth (rohu: 1.55% body weight day^{-1}, common carp: 1.59) and the highest total fish production (3,532 kg ha^{-1} 137 day^{-1}). The effects were less pronounced when stocking 1 common carp m^{-2}. Rohu shifted from phytoplankton to zooplankton when the latter became more available in response to artificial feed addition.

◊ Chapter 4 investigated the effects of common carp and artificial feed addition on water quality parameters and on the accumulation of nutrient in different types of natural foods present in the ponds. In all treatments all water quality parameters remained favourable for fish growth. The overall nitrogen and phosphorus concentrations were highest in treatments with 0.5 common carp m^{-2} followed by treatments with 1 and 0 common carp m^{-2}, respectively. The oxygen concentration decreased with increasing common carp density. More nitrogen and phosphorus accumulated in fish, phytoplankton and zooplankton in treatments with 0.5 common carp m^{-2}, followed by treatments with 1 and 0 common carp m^{-2}, respectively. The nitrogen and phosphorus accumulation in sediment was lower in presence of common carp than in the common carp free treatments. Concentrations of all nitrogenous and phosphorus compounds were higher in fed than in non-fed ponds except for NO_2-N. A larger fraction of the input nutrients accumulated in all natural food types in non-fed than fed pond whereas the opposite were observed for the fraction of nutrients accumulating in the sediment.

- In the third part of this study (Chapter 5) the resting, grazing, swimming and social interactions of rohu and common carp were observed in purpose build tanks for the same treatments as applied in part 2. Rohu decreased intra-species and increased inter-species interactions with increasing common carp density. Presence of common carp reduced the resting time and increased swimming and grazing time of rohu near the tanks bottom. Rohu benefited from spending more time close to the bottom and wall in presence of common carp by ingesting more zooplankton, as in these areas zooplankton biomass was higher than in other parts of the tanks.

A general discussion of the experimental results is given in Chapter 6. Both addition of common carp and artificial feed affected the pond ecology, fish growth, total production and fish behaviour. Stocking 0.5 common carp m^{-2} was better than stocking 1 m^{-2}. The strength of this study is that it looked at the combined effects of stocking benthivorous fishes and feed addition considering water quality, natural food availability, feed intake and behaviour. Results show that behavioural observations are equally important as water quality and feed intake monitoring to explain synergism in polyculture. Similar studies, adding a third species, are recommended to further elucidate changes in the food web dynamics when synergism is observed in polyculture ponds.

Samenvatting

Voedselwebinteracties en nutriëntendynamiek in polycultuurvijvers

Kunstmatig voer en meststoffen zijn de belangrijkste nutriëntenbronnen voor de groei van vis in visteeltvijvers. Het grootste deel van de toegevoegde voedingsstoffen gaat verloren in het sediment, waar het niet langer beschikbaar is voor de productie van natuurlijk voedsel. Door de resuspensie van het sediment te vergroten door middel van de introductie van benthivore vissen, kan het nutriëntenverlies worden beperkt, omdat de voedingsstoffen worden geremobiliseerd vanuit het sediment. De effecten van de toevoeging van benthivore vissen en/of kunstmatig voer in bemeste vijvers zijn tot nu toe meestal afzonderlijk bestudeerd. Dit proefschrift richt zich daarom op de geïntegreerde studie van (interacterende) effecten van de toevoeging van kunstmatig voer en een benthivore vissoort op de algehele nutriëntendynamiek, vijverecologie, visgroei en –productie in polycultuurvijvers. Hiervoor gebruikten we rohu (*Labeo rohita* Hamilton) en karper (*Cyprinus carpio* L.) - twee karperachtige vissen - , omdat de toepassing van de combinatie van rohu en karper in polycultuur populair wordt in Bangladesh. Karper is benthivoor en woelt het sediment op, wat leidt tot resuspensie van nutriënten, terwijl rohu een efficiënte planktoneter is. Drie dichtheden van karper (0, 0,5 en 1 individu m^{-2}) werden toegepast bij een vaste dichtheid van rohu (3 individuen m^{-2}), in vijvers met en zonder toevoeging van kunstmatig voer. Het doel van deze studie was om te kwantificeren wat de effecten van de toevoeging van voer en karper en hun interacties waren op de waterkwaliteit, de nutriëntenaccumulatie en de productie van natuurlijk voedsel en op het visgedrag, de visgroei en de visopbrengst. Bovendien probeerden we te bepalen of er een optimale karperdichtheid zou kunnen zijn voor de totale visproductie in vijvers met rohu.

Dit proefschrift is verdeeld in drie delen. Het eerste deel was een literatuurstudie over de polycultuur van karperachtigen en de rol van karper en kunstmatig voer op biotische en abiotische onderdelen van vijvers en meren. Het tweede deel analyseerde veranderingen van verscheidene biotische en abiotische onderdelen en de synergistische effecten die werden veroorzaakt door de rohu - karper combinaties. Het derde deel toonde veranderingen in zwem- en graasgedrag en de sociale interacties tussen rohu en karper.

- Het eerste deel van deze studie (hoofdstuk 1) richtte zich op de positieve en negatieve effecten van benthivore vissen op de beschikbaarheid van nutriënten en natuurlijk voedsel. Het bleek dat benthivore vissen de mineralisatie van organische stof, het vrijkomen van nutriënten vanuit het sediment naar de waterkolom en de primaire productie bevorderen. De dichtheid van benthivore vissen beïnvloedde de troebelheid van het water en de graasdruk op natuurlijk voedsel, welke op hun beurt de beschikbaarheid van natuurlijk voedsel beïnvloedden. De dichtheid van benthivore vis zal daarom de algehele prestatie van polycultuursystemen beïnvloeden.

- Deel twee bestaat uit de hoofdstukken 2, 3 en 4.

◊ Hoofdstuk 2 verkende de verbanden tussen waterkwaliteit, de verschillende beschikbare voedselbronnen in het water, de samenstelling van het dieet van de vis en visgroei en –productie. Karper vergrootte de biologische beschikbaarheid van stikstof en fosfor, wat nog werd versterkt door de toevoeging van kunstmatig voer. De effecten van karper waren sterker in de behandelingen met 0,5 dan in die met 1 karper m^{-2}. De PO_4-P concentratie was sterk gecorreleerd met de biomassa van fytoplankton en zoöplankton. Een van de belangrijkste bevindingen was dat de groei van rohu het best werd verklaard door de opname van natuurlijk voedsel, terwijl karpergroei het best door de toevoeging van kunstmatig voer werd verklaard. Bovendien was deze laatste negatief gecorreleerd met de opname van natuurlijk voedsel. De resultaten gaven aan dat karper op een directe manier profiteerde van de toevoeging van visvoer terwijl rohu daar indirect baat bij had doordat het bemestende effect van het visvoer de beschikbaarheid van natuurlijk voedsel vergrootte.

◊ In hoofdstuk 3 werden de effecten van verschillende dichtheden van karper (0, 0,5 en 1 individu m^{-2}) op de beschikbaarheid van natuurlijk voedsel, de opname van natuurlijk voedsel en visgroei vergeleken onder gevoerde en ongevoerde omstandigheden. Een dichtheid van 0,5 karper m^{-2} resulteerde in de hoogste beschikbaarheid van natuurlijk voedsel, de hoogste opname van natuurlijk voedsel, de hoogste visgroei (rohu: 1,55% lichaamsgewicht dag^{-1}, karper: 1,59) en de hoogste totale visproductie (3532 kg ha^{-1} 137 dag^{-1}). Deze effecten waren minder uitgesproken bij een dichtheid van 1 karper m^{-2}. Wanneer visvoer werd toegevoegd en daardoor de hoeveelheid beschikbaar zoöplankton toenam, nam de hoeveelheid zoöplankton in het dieet van rohu toe ten koste van fytoplankton.

◊ Hoofdstuk 4 ging in op de effecten van het uitzetten van karpers en de toevoeging van kunstmatig visvoer op de waterkwaliteit en op de nutriëntenaccumulatie in de verschillende natuurlijke voedselbronnen in de vijvers. In alle behandelingen bleven de waterkwaliteitsparameters gunstig voor visgroei. De totale stikstof- en fosforconcentraties waren het hoogst in behandelingen met 0,5 karper m^{-2}, gevolgd door de behandelingen met respectievelijk 1 en 0 karper m^{-2}. De zuurstofconcentratie nam af met een toenemende dichtheid van karper. De stikstof- en fosforaccumulatie in vis, fytoplankton en zoöplankton was het grootst in behandelingen met 0,5 karper m^{-2}, gevolgd door de behandelingen met respectievelijk 1 en 0 karper m^{-2}. De stikstof- en fosforaccumulatie in het sediment was lager in de aanwezigheid dan in de afwezigheid van karper. De concentraties van alle stikstof- en fosforcomponenten was hoger in gevoerde dan in ongevoerde vijvers, met uitzondering van de NO_2-N-concentratie. In de ongevoerde vijvers was de relatieve nutriëntenaccumulatie in de natuurlijke voedselbronnen groter dan in de vijvers waaraan visvoer werd toegevoegd. Het omgekeerde werd waargenomen voor de relatieve nutriëntenaccumulatie in het sediment.

- In het derde gedeelte van deze studie (hoofdstuk 5) werd het rust-, graas- en zwemgedrag en van rohu en karper geobserveerd en ook hun intra- en interspecifieke sociale interacties. Het gedrag werd geobserveerd in aquaria die dezelfde behandelingen ondergingen als beschreven in deel 2. De intraspecifieke interacties van rohu verminderden met een hogere dichtheid van karper, terwijl de interspecifieke interacties toenamen. De aanwezigheid van karper verminderde de rusttijd en verhoogde de zwem- en graastijd van rohu in de buurt van de aquariumbodem. In de aanwezigheid van karper profiteerde rohu ervan om langer in de buurt van de bodem of wanden van de aquaria te zijn, waar meer zoöplankton aanwezig was dan in andere delen van de aquaria, zodat ze hier meer zoöplankton konden opnemen.

In hoofdstuk 6 volgt een samenvattende discussie over de experimentele resultaten. Zowel het uitzetten van karper als het toevoegen van kunstmatig visvoer had een effect op de vijverecologie, visgroei, de totale visproductie en het gedrag. Het uitzetten van 0,5 karper m^{-2} was gunstiger dan het uitzetten van 1 karper m^{-2}. In deze studie werden de gecombineerde effecten van het uitzetten van benthivore vis en de toevoeging van visvoer op visvijvers op kwantitatieve manier onderzocht. Hierbij werden zowel waterkwaliteit, de beschikbaarheid van natuurlijk voedsel, voedselopname en gedrag, als groei en visproductie beschouwd. Deze geïntegreerde benadering is de belangrijkste bijdrage van deze studie aan onze kennis van polycultuursystemen. De resultaten tonen bovendien dat gedragsstudies even belangrijk zijn om synergie in polycultuur te begrijpen als het meten van de waterkwaliteit en de voedselopname. Vergelijkbare studies, waarbij een derde soort is toegevoegd worden aanbevolen om de dynamiek van het voedselweb te onderzoeken als synergie in polycultuur wordt waargenomen.

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M.M. Rahman
Wageningen
15 May, 2006

List of publications

Peer-reviewed articles

Azim, M.E., Verdegem, M.C.J., **Rahman, M.M.**, Wahab, M.A., van Dam A.A., Beveridge, M.C.M., 2002. Evaluation of polyculture of Indian major carps in periphyton-based ponds. Aquaculture 213,131-149.

Rahman, M.M., Verdegem, M.C.J., Nagelkerke, L.A.J., Wahab, M.A., Milstein, A., Verreth, J.A.J., 2006. Growth, production and food preference of rohu *Labeo rohita* (H.) in onoculture and in polyculture with common carp *Cyprinus carpio* (L.) under fed and non-fed ponds. Aquaculture (in press).

Rahman, M.M., Verdegem, M.C.J., Nagelkerke, L.A.J., Wahab, M.A., Milstein, A., Verreth, J.A.J. Effects of common carp *Cyprinus carpio* (L.) addition and feeding on pond water quality and nutrient accumulation in rohu *Labeo rohita* (Hamilton) ponds. Submitted.

Rahman, M.M., Verdegem, M.C.J., Nagelkerke, L.A.J., Wahab, M.A., Verreth, J.A.J. Relationships among water quality, food resources, fish diet and fish growth in polyculture ponds: a multivariate approach. Submitted.

Rahman, M.M., Verdegem, M.C.J., Nagelkerke, L.A.J., Wahab, M.A., Verreth, J.A.J. Swimming, grazing and social behaviour of rohu *Labeo rohita* (Hamilton) and common carp *Cyprinus carpio* (L.) in tanks under fed and non-fed conditions. Submitted.

Rahman, M.M., Verdegem, M.C.J., Nagelkerke, L.A.J., Wahab, M.A. Effects of day and night on swimming, grazing and social behaviours of rohu *Labeo rohita* (Hamilton) and common carp *Cyprinus carpio* (L.) in the simulated pond. Ready to submit.

Conference and Symposium contributions

Rahman, M.M., Verdegem, M.C.J., Wahab, M.A., Verreth, J.A.J., 2004. Effects of common carp *Cyprinus carpio* on growth, production and food habit of rohu *Labeo rohita* in fertilized or in fertilized and fed ponds. European Aquaculture Society. Special publication No. 34. Biotechnologies for quality, Barcelona, Spain, EAS (pp. 604).

Rahman, M.M., Verdegem, M.C.J., Wahab, M.A., 2004. Food preference of common carp *Cyprinus carpio* and rohu *Labeo rohita* in monoculture and polyculture: shifts in grazing on plankton under fed and unfed conditions. 7th Asian Fisheries Forum. 30 Nov.-4 Dec. 2004, Penang, Malaysia, AFF (pp. 302).

Rahman, M.M., Verdegem, M.C.J., 2005. Effects of supplementary feed on food preference of rohu *Labeo rohita* in mono and polyculture systems. World Aquaculture Society. Aquaculture America, January 200, New Orleans, USA. WAS (pp. 348).

Rahman, M.M., Verdegem, M.C.J., Nagelkerke, L.A.J., Verreth, J.A.J., 2005. Retention efficiency of nitrogen and phosphorous in semi-intensive mono and polyculture systems: very significant difference between fed and unfed ponds. WIAS science day, Wageningen, WAPS (pp. 17).

Rahman, M.M., Verdegem, M.C.J., Wahab, M.A., Verreth, J.A.J., 2005. Nitrogen and Phosphorous losses in semi-intensive mono and polyculture systems: very significant difference between fed and unfed ponds. World Aquaculture Society. World Aquaculture, May 9-13, 2005, Bali, Indonesia, WAS (pp. 512).

Rahman, M.M., Verdegem, M.C.J., Nagelkerke, L.A.J., Wahab, M.A., Milstein, A., Verreth, J.A.J., 2006. Effects of common carp additon to rohu ponds on food availability, fish behavior, fish production and waste discharge. Linking tradition and technology highest quality for the consumer. Firenze, Italy, WAS.

Rahman, M.M., Verdegem, M.C.J., 2006. Multi-species fish pond and nutrient balance. Symposium on Fish pond in farming systems. Can Tho, Vietnam. Wageningen Academic publishers (in press).

Training and Supervision Plan

Training and Supervision Plan **Graduate School WIAS**

Name PhD student	Mohammad Mustafizur Rahman	
Group	Aquaculture and Fisheries	
Daily supervisors	Marc C.J. Verdegem, Leo A.J. Nagelkerke	
Supervisor	Johan A.J. Verreth	
Project term	from 22.03.2002	until 14.06.2006
Submitted	date 21.03.2006	first plan / midterm / **certificate**

EDUCATION AND TRAINING (minimum 21cp, maximum 42 cp)

The Basic Package (minimum 2 cp)	year	cp*
• WIAS Introduction Course, Wageningen, Netherlands, January 20-24	2003	1.0
• Course on Philosophy of Science and Ethics, Wageningen, Netherlands, March 4-April 1	2004	1.0
Subtotal Basic Package		**2.0**

Scientific Exposure (conferences, seminars and presentations, minimum 5 cp)	year	cp
International conferences *(minimum 2 cp)*		
• Aquaculture Europe 2004 , Barcelona, Spain, October 21-23	2004	0.6
• 7th Asian Fisheries Forum, Malaysia, November 30-December 4	2004	1.0
• Aquaculture America 2005, USA, January 17-20	2005	0.8
• World Aquaculture 2005, Indonesia, May 9-13	2005	1.0
Seminars and workshops		
• Pond live workshop, Thailand, December 15-17	2003	0.6
• Challenges for Mediterranean Aquaculture, Barcelona, Spain, October 20	2004	0.2
• Pond live workshop, Malyasia, November 28-29	2004	0.4
• WIAS Science day	2004-2006	0.6
• Workshop on the recovery and artificial reproduction of eel. Bilthoven, Netherlands. March 14	2006	0.2
• Symposium on Fish Ponds in farming Systems. Can Tho, Vietnam. April 24-29	2006	1.2
Presentations *(minimum 4 original presentations of which at least 1 oral, 0.5 cp each)*		
• Oral Presentation in Pond live workshop, Thailand, December 15-17	2003	0.5
• Oral Presentation, Pond live workshop, Malyasia, Malyasia, November 28-29	2004	0.5
• Oral presentation, Aquaculture Europe 2004, Barcelona, Spain, October 21-2	2004	0.5
• Oral presentation, 7th Asian Fisheries Forum , Malaysia, November 30-December 4	2004	0.5
• Oral presentation, Aquaculture America 2005, USA, January 17-20	2005	0.5
• Oral presentation, World Aquaculture 2005, Indonesia, May 9-13	2005	0.5
• Oral presentation, WIAS Science Day	2005	0.5
• Oral presentation, Symposium on Fish Ponds in farming Systems. Can Tho, Vietnam. April 24-29	2006	0.5
Subtotal International Exposure		**10.6**

In-Depth Studies (minimum 4 cp)	year	cp
Disciplinary and interdisciplinary courses		
• Estuarine Ecology, Yerseke, Netherlands, May 10-14	2004	1.0
• Environmental Issue of Marine Fish Farming in the Mediterranean, Crete, Greece, Sep 1, 03 – Aug 16, 04.	2004	6.3
• Ecophysiology of the gastrointestinal tract, Wageningen, Netherlands, February 28-March 3	2005	1.0
• Aquaculture and the Environment, HAKI, Szarvas, Hungary, August 22-27	2005	1.2

Advanced statistics courses		
• Design of Animal Experiments, Wageningen, Netherlands, November 15-17	2002	0.6
Subtotal In-Depth Studies		**10.1**
Professional Skills Support Courses (minimum 2 cp)	year	cp
• Techniques for Writing and Presenting a Scientific paper, Wageningen, July 2-5	2002	0.8
• Project and Time Management, Wageningen, Netherlands, March-May	2004	1.0
• Career Perpective, Wageningen, Netherlands, February 1, 8, 9, 16, 22 and 23	2005	1.0
Subtotal Professional Skills Support Courses		**2.8**
Research Skills Training (apart from carrying out the PhD project, optional)	year	cp
• Preparing own PhD research proposal (optional, maximum 4 cp)		4.0
Subtotal Research Skills Training		**4.0**
Didactic Skills Training (optional)	year	cp
Supervising MSc thesis *(5% of the cp of the thesis)*		
• MSc theses (Richard Opio) on Interspecific interactions between common carp (*Cyprinus carpio*) and Rohu (*Labeo rohita*) in semi intensive pond polyculture	2005	1.3
Subtotal Didactic Skills Training		**1.3**
Education and Training Total		**30.8**

*One credit point (cp) equals a study load of approximately 40 hours

About the author

Mohammad Mustafizur Rahman was born on 8th December, 1974 in Mymensingh, Bangladesh. He is the eldest son of Md. Kalam Ali and Mrs. Rahima Akter. He completed his primary education at the Shayadgram Primary School, secondary education (SSC) at the Kheruazani High School and higher secondary education (HSC) at the Ananda Mohan College. He was enrolled at the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh in 1994 and obtained BSc Fisheies (Honors) degree in 1996. In 2000, he finalized his Master of Science (MS) degree in Fisheries Management at the same University. He obtained first class in all the degrees.

He worked in different research projects in different times, some of them are:

- EC funded research project on “The potential of periphyton-based aquaculture systems in south Asia” during June, 1999 to December, 2000.
- NORAD funded research project on “Environmental and Socio-economic Impacts of Shrimp Farming in Bangladesh” during January to July, 2001.
- USAID funded research project on "On-Station and On-Farm Trials of Different Fertilization Regimes Used in Bangladesh" during July 2001 to March 2002.

He started his PhD work on ‘Food Web Interactions and Nutrients Dynamics in Polyculture Ponds’ in March 2002 under the framework of an EC–funded INCO-pondlive programme. Wageningen University awarded him the full time Scholarship to study at the Wageningen University, the Netherlands to obtain PhD degree.

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Front: Conceptual model of major different trophic relations in differents common carp densities in rohu ponds (width of arrows towards similar direction among different trophic relations represent relative importance of effects; size of the same species of fish represents growth performance; number of the planktons and benthic macroinvertebrates indicate the relative abundance)

Back: Experimental tanks

Note