EFFECT OF DIFFERENT MANAGEMENT REGIMES ON THE SURVIVAL AND GROWTH OF EXOTIC ORNAMENTAL FISH, KOI CARP (*CYPRINUS CARPIO* L.), UNDER TROPICAL CONDITIONS

THESIS SUBMITTED TO THE UNIVERSITY OF NORTH BENGAL
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Certificate

This is to certify that the Thesis entitled, "Effect of different management regimes on the survival and growth of exotic ornamental fish, koi carp (*Cyprinus carpio* L.), under tropical conditions" is based on the original investigative study performed by Mr. Prithwiraj Jha, M.Sc., under my supervision, and that neither this Thesis, nor any part of it has been submitted for any degree or any other academic award anywhere before.

Mr. Jha has fulfilled the requirements of the degree of Doctor of Philosophy in Science (Zoology) of the University of North Bengal. He is conversant with techniques and literature cited in the dissertation and has carried out the work thoroughly. In character and disposition, Mr. Jha is fit to submit the Thesis for Ph.D. degree.

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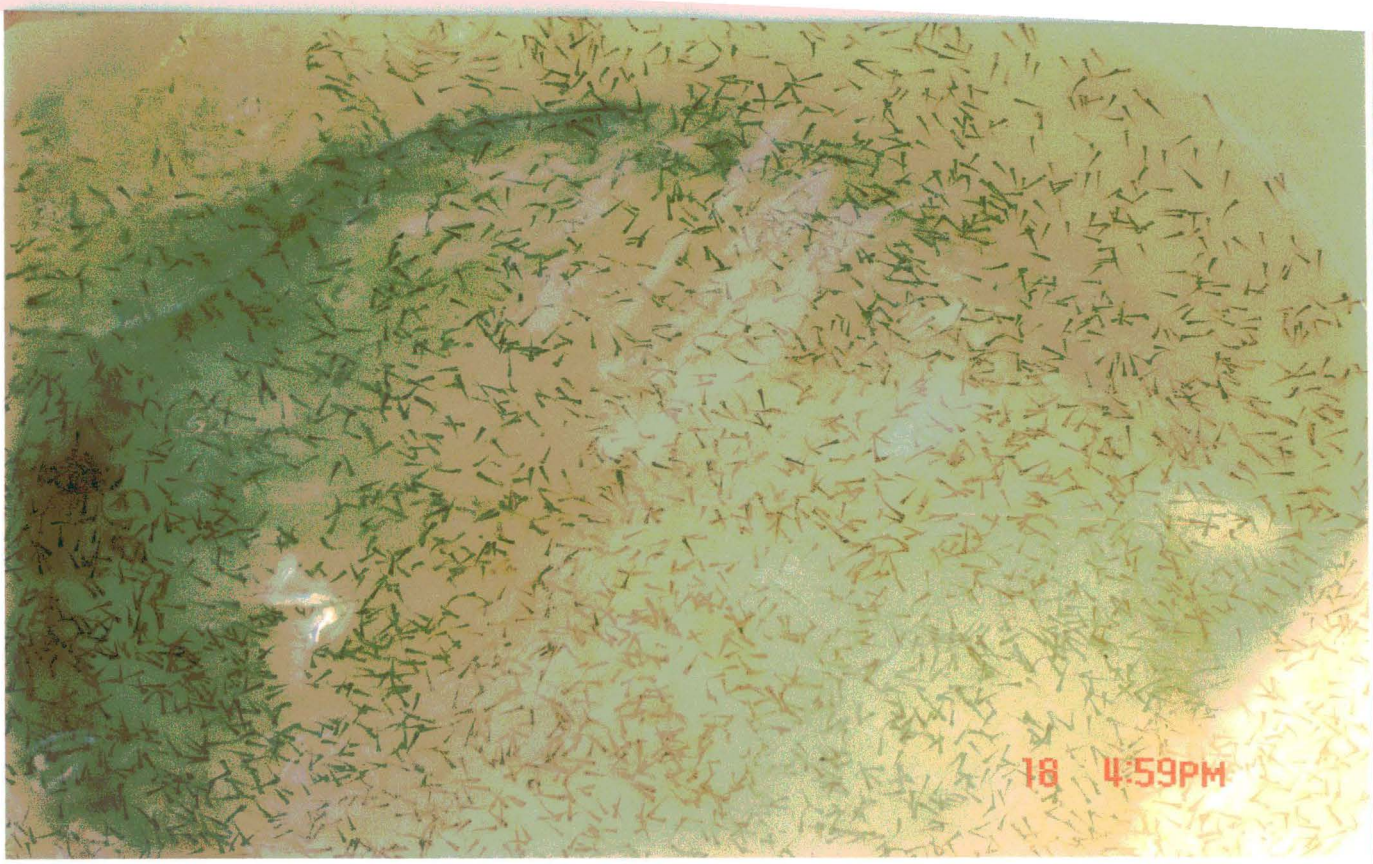

Dedicated to my mother

STATUTORY DECLARATIONS

Name of the Candidate	:	Prithwiraj Jha
Title of the Thesis	:	Effect of different management regimes on the survival and growth of exotic ornamental fish, koi carp (*Cyprinus carpio* L.), under tropical conditions
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Overwhelmed by the excitement of writing this section, which, in spite of its location, is attended to at the end of the writing process, I browsed through the acknowledgments of other dissertations. A vast majority of them impressed upon me the time-honoured tradition of beginning this section with the line, *This thesis would not have been possible without.....*; I was amused. It reminded me of a hackneyed line that the hero of mainstream Hindi movies almost unerringly blurts out to the heroine in the very precious moments between clobbering an entire army of hoodlums, *It is not possible for me to live without you!* However, even in the movies, the heroes seem to live on. Our *will to explore*, arguably, rivals our *will to exist*. It is quite possible that my Thesis would not come into existence under certain contingencies, but what could be a very probable one? I hope there weren't any.

However, *this* Thesis would not have been possible without the invaluable mentorship of Dr. Sudip Barat. Over the past six years of my interaction with him, I have developed a deep respect for his inhuman ability to tolerate harangues on unbaked (sometimes very crude) ideas, maintain an encouraging smile through out, and respond; *That's very interesting, but I think you are not eyeing the Noble Prize at present, simplify things a bit, may be you should try this out.* His favourite line with his students is, *Call me anytime!* – and he means it. He possesses the art of judging merits and demerits of a work, when coupled to his precision in formulating ideas on how to improve the experimental procedures and methodology, makes him a formidable thinker to challenge, and an enviable mentor to emulate. He encouraged me to discuss on various aspects of my research with other experts and develop a broad perspective. He is caring of his students' as a gardener is of his flowering plants, and his affection is untainted by superficiality.

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Thy infinite gifts come to me only on these very small hands of mine,
Ages pass, and still thou pourest, and still there is room to fill.

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Prithwiraj Jha.

(Prithwiraj Jha)

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Jha P., Sarkar K. and Barat S. (2004) Effect of different application rates of cowdung and poultry excreta on water quality and growth of ornamental carp, *Cyprinus carpio* vr. *koi*, in concrete tanks. ***Turkish Journal of Fisheries and Aquatic Sciences***, 4(1): 17 - 22.

Jha P. and Barat S. (2005) Effect of water exchange on water quality and the production of ornamental carp (*Cyprinus carpio* var. *koi* L.) in concrete tanks manured with poultry excreta. ***Archives of Polish Fisheries***, 13(1): 77 - 90.

Jha P. and Barat S. (2005) The effect of stocking density on growth, survival rate, and number of marketable fish produced of koi carps, *Cyprinus carpio* vr. *koi*, in concrete tanks. ***Journal of Applied Aquaculture***, 17(3): 89 - 102.

Jha P. and Barat S. (2005) Management induced changes in food selection, growth and survival of koi carp, *Cyprinus carpio* var. *koi* L., in tropical ponds. ***The Israeli Journal of Aquaculture - Bamidgeh***, 57(2): 115 - 124.

Jha P., Jha S., Pal B.C. and Barat S. (2005) Behavioural responses of two popular ornamental carps, *Cyprinus carpio* L. and *Carassius auratus* (L.) to monoculture and polyculture conditions in aquaria. ***Acta Ichthyologica et Piscatoria***, 35(2): 133 - 137.

Jha P., Sarkar K. and Barat S. (2006) Comparison of food selection and growth performance of koi carp, *Cyprinus carpio* L., and goldfish, *Carassius auratus* (L.) in mono- and polyculture rearing in tropical ponds. ***Aquaculture Research***, 37(4): 389 - 397.

Jha P., Barat S. and Sarkar K. (2006) Comparative effect of live-food and manured treatments on water quality and production of ornamental carp, *Cyprinus carpio* var. *koi* L., during winter, summer, monsoon and post monsoon growout experiments in concrete tanks. ***Journal of Applied Ichthyology***, IN PRESS.

Jha P., Barat S. and Nayak C.R. (2006) A comparison of growth, survival rate and number of marketable koi carp produced under different management regimes in earthen ponds and concrete tanks. ***Aquaculture International***, IN PRESS.

Jha P., Barat S. and Nayak C.R. (2006) Fish production, water quality and bacteriological parameters of koi carp ponds under live-food and manure based management regimes. *Manuscript in Communication.*

List of Abbreviations

ANOVA	Analysis of Variance
APHA	American Public Health Association
ASATM	Arc Sin Angular Transformation Method
BOD	Biological Oxygen Demand
C	Control
CD	Cow dung
cfus	Colony Forming Units
cm	Centimeter
^{0}C	Degree Celsius
DO	Dissolved Oxygen
FCR	Food Conversion Rate
g	Gram
G	Goldfish
GI pipe	Galvanized Iron pipe
ha	Hectare
K	Koi carp
kg	Kilogram
L	Litre
lb	Pound
LF	Live-food
m	Meter
mg	Milligram
mL	Millilitre
mmhos	Millimhos
No.	Number
P	Probability
pH	Hydrogen Ion Concentration
PM	Poultry manure
r	Simple correlation or regression
SE	Standard Error
SGR	Specific Growth Rate
THSD test	Tukey's Honestly Significant Difference test

1. Introduction

1.1. General introduction

Ornamental fish can be defined as attractive and colourful fish of peaceful nature that are kept as pets in confined spaces of an aquarium or a garden pool with the purpose of enjoying their beauty for fun and fancy (Dey, 1996). The soothing effect of aquariums in helping to relieve some of the pressures of modern urban life have helped, in part, to make ornamental fish keeping a popular pastime and an important commercial activity. Fish keeping is today the world's most popular hobby after photography and ornamental fish are the most popular pets in the world (Singh, 2005). As such, production of animals for the aquarium hobbyist trade is rapidly increasing. The ornamental aquatic sector has shown overall expansion since 1985, and until 1996, the annual growth rate in the world trade of ornamental fish was about 14% (Dawes, 2002). Although there was a periodic depression in the late 1990's (Olivier, 2000), the new millennium has ushered very promising resurgence. A conservative estimate of the annual wholesale value of the current world trade puts it at more than US $ 1 billion. Some 1.5 billion fish are traded yearly with a retail value of at least US $ 6 billion (Singh, 2005). The value of the entire industry, when non-exported product, wages, retail sales and associated materials are considered has been estimated at US $ 15 billion (Subasinghe, 2005).

Unfortunately, the ornamental aquatic sector in India is still in a state of hibernation. The country exported about US $ 0.7 million worth of ornamental fish during 2003-04 (Bojan, 2005), which is virtually negligible in the international scenario. The domestic market, however, is rapidly expanding, with a current turn over of Rupees 150 million (US $ 3.5 million) and an annual growth rate of 20% (Bhattacharjya and Choudhury, 2004; Dehadrai, 2004). There is a great potential for expansion of ornamental fish trade, both in India and abroad.

About 70% of India's population lives in villages and 90% of its rural population depend on agriculture and allied activities like fish capture/aquaculture for their livelihood (Radheyshyam, 2001). With her enormous natural and human resources, which could be mobilized for aquaculture activities, India could become a major player in the international ornamental fish market through a properly planned approach. Establishment of an ornamental fish culture industry has long been felt to be one means to diversify the aquaculture sector in India, and would enable Indian producers to win

market share, both locally and internationally. According to an estimate by Swain and Jena (2002), the country has the potential to increase the export of ornamental fish to about US $ 30 million every year within the next decade. The key to realizing this opportunity is the development of aquaculture technology suited to Indian conditions. Diversification of aquaculture has been given a focused attention in the country's 10th Five Year Plan with special emphasis on ornamental fish culture (Tripathi, 2004). Thus a new dimension is added to fisheries research in the country and researchers need to document commercial production techniques of various ornamental fish species to meet the burgeoning demand.

1.2. Key areas identified for research

The ornamental fish industry in India can be divided into two major sectors: (i) exotic fresh water species (this is the largest sector and includes both coldwater and tropical fish); and (ii) indigenous fresh water species (includes both coldwater and tropical fish of Indian origin) (Jha and Barat, 2005). There is also a very small sector catering to marine ornamental species. The indigenous ornamental fish market is completely capture based, while the exotic species are cultured. However, the production of exotic ornamental fish is mostly restricted to a few metropolitan cities like Kolkata, Mumbai and Chennai (Swain, 2004), and absence of proper technology has acted as a barrier for expansion of ornamental fish culture throughout the country.

A visit to some of the top ornamental fish production units of West Bengal state in India during 2001, and discussions with the producers/farmers threw open some avenues that could be researched upon. Further deliberations with aquaculture experts resulted in pointing out specific questions that were to be dealt during the study.

It was felt, that the culture technology for exotic ornamental fish under tropical conditions in India needs to be standardized. Different types of management regimes would be systematically tried to find suitable culture conditions for optimal fish production. Koi carp (*Cyprinus carpio*) was selected as a model species for the study. Also, the fact that the culture of koi carp is rapidly growing in India, and farmers were keen to know the culture techniques for the fish played an important role in its selection. Another reason for the selection of this fish was the easy availability of koi carp larvae throughout the year in Jalpaiguri district of North Bengal, the place of our study.

A common approach for increasing fish production in ponds is the direct application of fertilizer, which enhances production of plankton, a natural food item for fish (Jhingran, 1991; Chakrabarti and Jana, 1998; Ansa and Jiya, 2002; Kadri and Emmanuel, 2003). Pond fertilization practices using animal wastes are widely used in many countries to sustain productivity at low cost (Pekar and Olah, 1990; Fermin, 2001; Gupta and Noble, 2001; Tripathi and Sharma, 2001; Majumder *et al.*, 2002). Manure usage at different rates may significantly influence water quality and assist in defining the optimal conditions for continuous culture of plankton. However, the use of organic manure in ornamental carp production systems has not been documented and the application rate needs to be standardized.

Ornamental fish culture in India is practiced in particularly two types of culture systems: earthen ponds and concrete tanks. Since most farmers in India cannot afford aeration facilities, water exchange is used as an alternative to maintain water quality in the tanks. It was felt, that the effect of different water exchange regimes on water quality and koi carp production in organically manured tanks should be documented. To supply the growing market, fish farmers need to keep fish at the highest sustainable stocking densities to produce a large number of fish, hence the stocking density for koi carp also needs to be optimized.

The koi carp has a market for individuals above 4 g, and require only about 10 - 12 weeks of growout to attain the marketable size. As such, ornamental fish producers have the opportunity to harvest three to four crops throughout the year (during different seasons). In the tropical plain lands of North Bengal, pond water temperature falls below 20^{0}C for only three months in a year, that is, mid-November to mid-February. There are several reports on the influence of water temperature on the feeding activity, metabolism, growth and production of fish (Weatherley, 1990; Shrestha, 1999). It was felt, that the influence of the growth period or season on koi carp production has not been documented and requires detailed investigation.

There are some reports on organic manuring leading to depletion of dissolved oxygen, high biological and chemical oxygen demand, and generation of H_2S, methane and ammonia (Boyd, 1982; Wong *et al.*, 1982; Singh *et al.*, 1991). The resultant stress can ultimately lead to exhaustion, disease and mortality in fish (Francis-Floyd, 1990). However,

without directly applying organic manures, if the benefits of organic manuring, namely, live plankton could be channeled to fish culture tanks or ponds from other sources, environmental conditions in the fish culture systems would not be reduced. Since ornamental fish ponds in India are much smaller compared to other aquaculture ponds (measuring about 7 m × 20 m, with an average depth of 0.6 - 1.0 m), there are more opportunities to control environmental conditions in ornamental fish ponds by employing similar management techniques. It was felt, that introduction of live zooplankton could be explored as an alternate to direct organic manuring for increasing ornamental fish yields while avoiding water quality deterioration.

Any management applied would have a different effect on the interactions of water quality, phytoplankton and zooplankton, with respect to earthen ponds and concrete tanks. This could lead to differences in fish production in both systems. It was felt, that the effect of different management protocols on water quality, plankton abundance, and koi carp production in earthen ponds and concrete tanks should be compared.

The feeding habit and food preferences of koi carp in tropical conditions and its effect on fish production warrant proper documentation. The role of heterotrophic bacteria in the aquatic food web and its effect on fish yield are poorly documented (Moriarty, 1987). According to our information, there have been no research studies on the abundance of heterotrophic bacteria in ornamental fish ponds in India. Besides, freshwater fish in Indian ponds most commonly suffer from bacterial diseases such as, various kinds of skin ulcerations, albinoderma, erythroderma, furunculosis, and verticle-scale disease, primarily caused by *Aeromonas* sp. and *Pseudomonas* sp. (Das, 2004). Hence, bacteriological parameters, particularly the isolation and total counts of *Aeromonas* sp. and *Pseudomonas* sp. in koi carp ponds also demands detailed investigation.

One interesting aspect that regularly came up during the discussions was whether koi carp could be polycultured. Ornamental fish are mostly monocultured for reasons described later. Pond culture has presented the opportunity to polyculture ornamental carps, particularly, species like koi carp and goldfish, *Carassius auratus*, that have a similar marketable size (> 4.0 g) and require a similar culture period of 10 - 12 weeks to attain the marketable size, could be stocked together to optimize the utilization of available resources. However, further work on the impacts of polyculture on the overall culture

performance of each species is necessary. Behavioural studies on the interspecific interrelationships are one tool for evaluation. It was felt that the behavioural responses of koi carp and goldfish stocked in mono- and polyculture combinations should be compared to assess their behavioural compatibility. In addition, the growth and food selection of koi carp and goldfish raised in monoculture and polyculture combinations require detailed investigation.

Based on the above-mentioned needed avenues of research in ornamental fish farming, a research project entitled, "Effect of different management regimes on the survival and growth of exotic ornamental fish, koi carp (*Cyprinus carpio* L.), under tropical conditions", was initiated in February, 2002, to document the following objectives.

1.3. Objectives of the present investigation

- ✓ To study the effect of different application rates of organic manures (cow dung and poultry excreta) on the production of ornamental fish (koi carp).
- ✓ To document the effect of different water exchange regimes on koi carp production in organically manured tanks.
- ✓ To investigate the effect of different stocking densities on koi carp production.
- ✓ To explore the possibility of supplying exogenous zooplankton against direct organic manuring in fish culture systems in maintaining better culture environment and high koi carp production.
- ✓ To examine the seasonal influence on koi carp production.
- ✓ To compare koi carp production in earthen ponds and concrete tanks.
- ✓ To observe the feeding habit and food selection of koi carp.
- ✓ To monitor the growth responses of heterotrophic bacteria, along with the development of *Aeromonas* sp. and *Pseudomonas* sp. in koi carp ponds.
- ✓ To compare the behavioural responses of koi carp and goldfish stocked in mono- and polyculture combinations, for assessing their behavioural compatibility.
- ✓ To evaluate and compare the growth and food selection of koi carp and goldfish raised in mono- and polyculture combinations.

The nucleus of the present study, is therefore, likely to depict a better understanding on the cultural requirements of the koi carp (*Cyprinus carpio*) under tropical conditions.

2. Review of Literature

2.1. Introduction to ornamental fish rearing

Ornamental fish production for the aquarium industry is increasing steadily throughout the world. According to Singh (2005), about 750 fresh water species are traded, among which nearly 90% are from aquaculture and 10% are collected from the wild. According to trade statistics, Asian countries dominate the world export market. Out of the figure of US $ 189.5 million of total exports in 2002, Asian countries had a share of about 60%. Singapore was the world's largest exporter contributing about 22% of total exports (**Table 1**), followed by Malaysia (9.3%), the Czech Republic (7%) and Indonesia (6.7%).

Table 1 World trade in ornamental fish (2002): share of main exporting countries (adapted from Singh, 2005).

Country	Amount of export (in US $)	% of global trade
Singapore	41.46	21.88
Malaysia	17.56	9.27
Czech Republic	13.35	7.04
Indonesia	12.65	6.67
China	9.48	5.00
United States	8.38	4.42
Japan	8.33	4.39
Philippines	6.44	3.40
Peru	6.44	3.40
Sri Lanka	5.52	2.91
Others	59.88	31.60
Total	**189.49**	**100**

Although most of the ornamental fish are exported from countries in south-east Asia, there is little information pertaining to husbandry and culture management of ornamental fish species from these countries. One of the possible reasons could be the intense competition between different fish farms, where commercial production techniques are closely guarded by the producers in this increasingly knowledge-based industry. Tay and Tan (1976), and Tay (1977) reported on cage culture of ornamental fish in earthen ponds in Singapore. However, water scarcity in Singapore, where almost half of the water comes from neighboring countries (Tan, 1988), forced fish producers to limit unrestricted use of water. Fernando and Phang (1985) documented the culture techniques of guppy, *Poecilia reticulata*, in some fish farms of Singapore, where large,

shallow, cement tanks were used for breeding, grow-out of fry and juveniles, and stocking. In some farms, glass tanks were used for stocking and conditioning. However, every farm had at least one reservoir pond. Pig dung was added as fertilizer to the cement tanks and cage-net ponds in some farms to enhance the growth of natural food organisms. Fertilization of ponds with poultry excreta or pig dung has also been reported in the culture of other ornamental species (Tay and Tan, 1976). Ng *et al.* (1992) documented the water quality management in a tropical fish farm in Singapore through a discontinuous flow recirculation system, with daily 8% exchange of water. Over the years, in order to increase productivity for intensive farming, most farmers are increasingly using the water recycling system. The intension is to create an appropriate ecological and biological system, which serves to breed and farm fish in an efficient and controlled environment (Lee, 2005).

In Malaysia, significant developments in the ornamental fish culture industry took place in the 1980s, when fish farms were set up in different locations in the country (Dey, 2005). According to official statistics published by the Department of Fisheries, Malaysia, there seems to be a degree of specialization among different states in the production of fish. While producers in Penang specialize in discus, *Symphysodon discus*, farmers in Perak give emphasis to goldfish and koi carp, which is attributable to proper understanding of the culture requirements of each species and the environmental conditions of the respective state.

Compared to Asia, much more information is available on ornamental fish culture in the United States. Watson and Shireman (1996) documented ornamental fish production in Florida. Here, tropical fish are cultured primarily in outdoor earthen ponds. Relative to other aquaculture ponds, ornamental fish ponds are very small, averaging 7.62 m × 22.86 m, with a maximum depth of about 1.83 m. The pool is first pumped dry and hydrated lime is added as a sterilant. Organic fertilizers such as cottonseed meal are also added in the pond. An earlier report by Martin (1983) indicated that compared to larger ponds in Arkansas and Kentucky, ornamental fish production was higher in ponds that were smaller than 1 acre.

Tamaru *et al.* (1997) summarized the different culture systems employed in the production of ornamental fish in Hawaii. Traditionally, ornamental fish are produced in earthen ponds because the bottom soils support a healthy growth of plankton from

which the fish feed on. Besides, fish culture in ponds brings down construction costs. However, earthen ponds tend to have aquatic weed problems, and because of their large size are difficult to control. Fish culture in circular tanks allows for more effective measures in controlling the rearing management. Besides, plankton concentration in 'green water' tank culture systems is comparable to earthen ponds. Cage culture in large ponds falls somewhere in between tank and pond growout culture in terms of easy management and productivity, and serves as a cost effective way to diversify production. Asano *et al.* (2003) reported that ornamental fish are typically produced in culture tanks that exchange four tank volumes of water in a day. Since 1993, the Centre for Tropical and Subtropical Aquaculture in Hawaii have produced a series of user-friendly documentations on different aspects of culture of ornamental fish. Production protocols are available for the blue gourami, *Trichogaster trichopterus* (Cole *et al.*, 1997), tiger barb, *Capoeta tetrazona* (Tamaru *et al.*, 1997), serpae tetra, *Hyphessobrycon serpae* (Cole and Haring, 1999), lemon tetra, *Hyphessobrycon pulchripinnis* (Cole *et al.*, 1999) and swordtail, *Xiphophorus helleri* (Tamaru *et al.*, 2001).

Some literature on cultural conditions of ornamental species is available from other regions of the world. In Israel, the culture of ornamental cyprinids, mainly goldfish and koi carp are growing rapidly. Large scale fish production involves fry stocking in earthen ponds where large mortality have been reported before the fish reach 1 g size (Feldlite and Milstein, 1999). In South Africa, experimental tank culture of swordtail with water exchange rates of 1.5 - 2 theoretical turnovers per hour yielded growth rates similar or better than those found under commercial conditions (Kruger, 1995; Olivier and Kaiser, 1997). In another study, survival rate and length increment of swordtail were unaffected by water exchange rates but faster water exchange promoted a more uniform size of fish (Kaiser and Jones, 1998). Vine and Kaiser (1994) reported good production of guppy under high stocking densities in culture tanks where water was replaced 4 - 5 times per hour. Kaiser *et al.* (1998) documented the diurnal water quality fluctuations in a closed recirculating system for the culture of guppy and concluded that a water exchange rate of six turnovers per hour was required to reduce the occurrence of ammonia peaks. In Europe, ornamental fish business is seasonal, with a focus on garden ponds in summer and on aquaria in the winter (Postoma *et al.*, 2004). The Czech Republic is the largest producer of ornamental fish in Europe. Typical fish productions in the Czech Republic

are small basement operations. A preliminary survey showed that some producers even operate from small rooms measuring about 16 m^2, producing nearly 12000 fish per month, using modern techniques (Rana, 2004).

In India, few cities like Kolkata, Mumbai and Chennai are the most important production centres for ornamental fish (Swain, 2004). According to Dehadrai (2004), there are about 300 full time producers and 600 part time producers of ornamental fish throughout the country (**Table 2**). Nearly 90% of total export of ornamental fish from India is conducted through Kolkata airport in West Bengal (Biswas and Lepcha, 2004). According to Swain *et al.* (2003) and Mukherjee (2004), export is limited to captured indigenous fish (85%), and cultured exotic ornamental species contribute to only 15% of the total exports. One of the reasons could be that although the exotic species have been farmed on a large scale for many years, little scientific research has been conducted on their culture and trade requirements. According to trade statistics, exotic species are in most demand, both in the global (Singh, 2005) and domestic markets (Biswas and Lepcha, 2004), and proper emphasis should be given to understanding their culture requirements under tropical conditions.

Table 2 Information on number of ornamental fish units and people involved with the trade in India (adapted from Dehadrai, 2004).

Type of business	Number of units
Regular ornamental fish farms	4
Full time breeders and growers	300
Part time breeders and growers	600
Aquarium shops (large)	250
Aquarium shops (small)	1500
Ornamental fish exporters	20

2.2. Food requirements of ornamental fish

In aquaculture, food is considered the most powerful among environmental variables affecting growth and metabolism (Kinne, 1962; Beamish and Dickie, 1967; Miller *et al.*, 1988; Kaiser *et al.*, 1997; Bunnell *et al.*, 2003). A modest amount of literature is available on the nutrition and food requirements of some popular ornamental fish species.

Nayadu (1975), Dussault and Kramer (1971), and Dahlgren (1980) documented the omnivorous feeding habit of guppy, with a varied food preference of small invertebrates, insect larvae, algae and other plant material. Fernando and Phang (1985) reported the application of formulated diets in fish farms of Singapore, which included milk powder, wheat bran, wheat flour, fish meal, egg yolk, minced beef and ground dried shrimp. In some farms, supplemental live food consisting of water fleas and tubificiid worms were also added. Much emphasis is placed upon regular supplementation of diets with live food, including cladocerans such as *Daphnia* and *Moina*, and tubificiid worms (Fernando *et al.*, 1991). A dietary protein requirement of about 30 - 40% for the guppy was reported by Shim and Chua (1986). Harpaz *et al.* (2005) studied the effect of feeding guppy fry with different forms of commercial diets and concluded that the growth was considerably enhanced when the diet was presented in powdered form, compared to flakes.

Gut content analysis of the swordtail and platy, *Xiphophorus maculatus* revealed that both terrestrial and aquatic insect larvae are eaten along with phytoplankton and some micro algae (Arthington, 1989). However, the sailfin molly, *Poecilia latipinna* prefers a diet of more plant material (Dawes, 1991). Kruger *et al.* (2001 a) conducted a series of experiments to develop a diet suitable for early juvenile swordtail under intensive culture conditions and found that a diet with 45% protein content promoted growth rates and feed conversion. Supplementation of flakes with *Daphnia* resulted in improved growth rate, compared with only flake feed (Kruger *et al.*, 2001 b). Likewise, an increment in growth rate was reported in angelfish, *Pterophyllum scalare*, when *Artemia* was supplemented with a commercial trout diet (Degani, 1993). Tiger barb is generally considered omnivorous (Tamaru *et al.*, 1997). Investigations by Shiraishi *et al.* (1972) on gut contents of wild caught tiger barb showed phytoplankton, zooplankton, aquatic and terrestrial insect larvae, and plant tissue, with a strong preference for vegetative diet. The blue gourami is considered carnivorous, the natural diet being different species of invertebrates (Degani, 1990). For the pearl gourami, *Trichogaster leeri*, a dietary protein requirement between 26% and 36% was suggested by Degani and Gur (1992).

Studies on food requirements of ornamental cyprinids lay emphasis on the importance of live food. Martin (1983) reported on the supply of zooplankton to goldfish

ponds by some producers in the United States and suggested a dietary protein requirement of 38% to 45% for the goldfish. Abi-Ayad and Kestemont (1994) reported an increment of growth in goldfish fed with live *Artemia*, compared to fish fed with dry diet. However, Kaiser *et al.* (2003) suggested the use of decapsulated *Artemia* cysts, compared to live *Artemia*. The effectiveness of decapsulated *Artemia* cysts in promoting growth and survival of a number of ornamental species have also been documented by Lim *et al.* (2003). Lubzens *et al.* (1987) observed that goldfish fed with rotifer, *Brachionus plicatilis*, in combination with artificial feed, showed better growth than fish fed with dry diet only. Combination of greenwater infusoria (including mainly ciliates) with artificial diets yielded better growth of rainbow shark, *Epalzeorhynchos erythrurus*, compared to fish fed with artificial feed only (McGovern-Hopkins *et al.*, 2002). Studies on koi carp by Ako and Tamaru (1999) suggested that growth rate increases with the palatability of the diet. Appelbaum *et al.* (1986) and Van Damme *et al.* (1989) reported high mortality rates of koi carp larvae and juveniles when fed with artificial diets. Experiments by Lubzens *et al.* (1987) demonstrated that relative to dry diet, supply of *Brachionus plicatilis* improved survival and growth rate in koi carp.

Management strategies of fish husbandry are likely to influence species composition and abundance of live food in the environment (Diana *et al.*, 1991; Milstein *et al.*, 1995; Garg and Bhatnagar, 1996; Akpan and Okafor, 1997; Jakubas, 2002; Mischke and Zimba, 2004), which could in turn affect the food selection by the target fish species. Two distinct types of plankton feeding behaviour are distinguished: particulate feeding and filter feeding. In nature, switching from particulate to filter feeding behaviour is a function of various factors such as prey density and the size range of available prey (Lazzaro, 1987; Dewan *et al.*, 1991; Ushakumari and Aravindan, 1992; Xie, 1999; Serajuddin, 2000). Some planktonic organisms pass undigested through the gut of planktivorous fishes. Since the fish expends energy in the capture of prey and receives no energy through assimilation in return, the fish may recognize and avoid such undesirable prey organisms. Ivlev (1961) and Vinyard (1967) reported on slightly negative electivity towards ostracods by bleak, *Alburnus alburnus*, and bluegill, *Lepomis macrochirus*, respectively. However, detailed documentation pertaining to food selection of ornamental cyprinids under tropical conditions is sparse.

2.3. Stocking density of ornamental fish

To supply the growing market, fish farmers need to keep fish at the highest sustainable stocking densities to produce a large number of fish (Olivier and Kaiser, 1997). Knowing the optimal stock density is one of the basic factors of intensive fish culture. This density should be the resultant value of the environmental requirements of a given fish species and broadly understood economic efficiency (Holm *et al.*, 1990; Kuipers and Summerfelt, 1994; Szkudlarek and Zakes, 2002). Fish stocking density is the most sensitive factor determining the productivity of a culture system as it affects growth rate, size variation and mortality (Kaiser *et al.*, 1997).

While the effect of stocking density on growth and production of food fish species has undergone intensive investigations, little scientific research has been conducted on ornamental fish species. In comparison to food fish production, the densities at which ornamental fish have been kept are rather low. In Singapore, the stocking rate have been reported to be as low as 0.02 - 0.1 fish/ L (Ng *et al.*, 1992) to less then 0.3 fish/ L (Fernando and Phang, 1985). Among other literatures available, values range from 0.4 fish/ L in angelfish (Degani, 1993) and swordtail (Mondal *et al.*, 2004) to 0.5 fish/ L for the blue gourami (Cole *et al.*, 1997). The koi carp is traditionally cultured at a density of 0.25 fish/ L in Hawaii (Asano *et al.*, 2003). However, to our knowledge, there have not been any research studies on stocking rates for koi carp production under tropical conditions.

2.4. Polyculture of ornamental fish

Polyculture is the only possible way of simultaneously producing more than one fish species from the same rearing space (Papoutsoglou *et al.*, 1992; Papoutsoglou *et al.*, 2001). The principle of polyculture is based on the fact that cultured fish species feed on different levels of food chain and environment (Milstein *et al.*, 2002). The productivity of the aquatic system is thus increased by more efficiently utilizing ecological resources within the environment. Stocking two or more complimentary fish species can increase the maximum standing crop of a pond by allowing a wide range of available food items and the pond volume to be utilized (Lutz, 2003).

Most of the literature available on the husbandry of ornamental fish suggests monoculture (Fernando and Phang, 1985; Kestemont, 1995; Asano *et al.*, 2003; Kaiser *et al.*, 2003; McGovern-Hopkins *et al.*, 2003). One of the possible reasons could be the differences in the culture period for different fish species (Watson and Shireman, 1996). While food fish producers can sell any amount of fish harvested, ornamental fish are sold by number and have to be of a minimum size to be accepted in the market (Olivier and Kaiser, 1997). Some species have a market for small individuals, and the farmer may harvest the pond after only eight to ten weeks of growout. Others may require much longer culture periods. The extreme diversity of the industry prohibits gross generalizations in this area (Watson and Shireman, 1996). Another reason could be the scarcity of documentation pertaining to behavioural compatibility and interspecific interrelationships between different ornamental species.

2.5. Organic manuring in aquaculture

Organic manures are regarded as a composite class and contain almost all the essential nutrient elements (**Table 3**) required in a pond ecosystem (Pillay, 1993; Jana *et al.*, 2001). They are known to improve soil structure and fertility (Pillay, 1993). Being less expensive compared to chemical fertilizers, organic manures are traditionally applied to fish ponds to release inorganic nutrients which stimulate the growth of plankton (Moore 1985; Schoonbee and Prinsloo, 1988; Green *et al.*, 1990; Jhingran, 1991; Yadava and Garg, 1992; Edwards *et al.*, 1996; Garg and Bhatnagar, 1996; Mahboob and Sheri, 1997, Atay and Demir, 1998; Begum *et al.*, 2003), which form the nutritious and preferred food of many aquaculture species. In addition to their importance in the ornamental aquatic sector, zooplankton is also required as a first food for most species of food fish and contributes to faster larval growth and better survival (Prinsloo and Schoonbee, 1986; Rottmann *et al.*, 1991; Pillay, 1993; Adeyemo *et al.*, 1994; Welker *et al.*, 1994; Ludwig, 1999; Sharma and Chakrabarti, 1999; Al-Harbi and Siddique, 2001). Greater abundance of plankton supports larger populations of cultured fish species (Wurts, 2004). Lin *et al.* (1997) documented that under acidic soil conditions, fertilization with organic manure (poultry excreta) resulted in significantly increased plankton production compared to inorganic (NPK) fertilizers.

Table 3 Composition of fresh manure from various animal species (after Pillay, 1993).

Components	Mixed dung	Horse dung	Cattle dung	Sheep dung	Pig dung
Water	75.0	71.3	77.3	64.6	72.4
Organic matter	21.0	25.4	20.3	31.8	25.0
Total nitrogen (N)	0.50	0.58	0.45	0.83	0.45
Proteinic nitrogen	0.31	0.35	0.28	-	-
Ammoniacal nitrogen	0.15	0.19	0.14	-	0.20
Phosphorus (P_2O_5)	0.25	0.28	0.23	0.23	0.19
Potassium (K_2O)	0.60	0.63	0.50	0.67	0.60
Calcium (CaO)	0.35	0.21	0.40	0.33	0.18
Magnesium (MgO)	0.15	0.14	0.11	0.18	0.09
Sulphuric acid (SO_3^{2-})	0.10	0.07	0.06	0.15	0.08
Chlorine (Cl^-)	-	0.04	0.10	0.17	0.17
Silicic acid	-	1.77	0.85	1.47	1.08
Iron and aluminum sesquioxides (R_2O_3)	-	0.11	0.05	0.24	0.07

In India, cow dung is the most common organic manure applied to fish ponds and other animal wastes have not been systematically tried (Singh and Sharma, 1999). Perhaps the easy availability of cow dung in rural India has played a determining role in its wide use in aquaculture. Among other manures, chicken's is preferred worldwide because of its ready solubility and high level of nitrogen and phosphorus concentrations (Kapur and Lal, 1986; Knud-Hansen *et al.*, 1991; Singh and Sharma, 1999; Sevilleja *et al.*, 2001). With the rapid expansion of poultry husbandry during the last two decades, poultry excreta have become increasingly available in rural India. However, organic manures other than cow dung and poultry excreta are available only in pockets; hence, their utilization in aquaculture throughout India has a very limited possibility.

2.6. Effect of organic manuring on the aquatic food web

The purpose of pond fertilization is to augment fish production through autotrophic and heterotrophic pathways (Jana *et al.*, 2001). A series of complicated processes is involved between the input of manure and the ultimate output of fish in the pond ecosystem via material cycling, production of fish food organisms and energy flow (Xianzhen *et al.*, 1988; Green *et al.*, 1989; Schroeder *et al.*, 1990; Knud-Hansen and Batterson, 1994). Besides acting as the primary nutrient source, organic manure may also enhance fish production through detrital formation (Wohlfarth and Schroeder,

1979) and exert a definite impact upon the microbial-detrital food chain of the aquatic system (Rappaport and Sarig, 1978; Pillay, 1993). Radio isotopic tracer method has been employed to detect the different production pathways in manure based fish ponds (Pekar and Olah, 1998). Carbon isotope study indicated that in common carp, *Cyprinus carpio*, 50 - 70% of the carbon originated from manure food webs and 30 - 50% originated from micro algae, whereas, in crucian carp, *Carassius carassius*, only 22% carbon was contributed by the manure and 60 - 80% carbon originated from micro algal production (Xianzhen *et al.*, 1986).

The available organic pool in manured ponds is usually duplicated everyday via bacterial production (Schroeder, 1987). Heterotrophic microorganisms, necessitating some organic sources of carbon in addition to inorganic forms for growth, have a significant role in the decomposition of organic matter and production of particulate food materials from dissolved organics (Schroeder and Hepher, 1979; Jana and De, 1990). In the decomposition process, bacteria emerge as the first link between the living world and the abiotic factors (Pekar and Olah, 1990). Although actinomycetes and fungi are also known to take part in the decomposition process (Boyd, 1995), their role are much restricted compared to bacteria (Gaur *et al.*, 1995). The rate of decomposition of organic matter also varies depending upon its composition and the physico-chemical environment of the culture system. The rate of nutrient release from animal manure over time generally determines the fertilization schedule to be adopted in a given pond (Egna and Boyd, 1997). The availability of inorganic nutrients from poultry excreta was reported to be considerably higher than that from manures of pig, goat and cow (Kapur and Lal, 1986).

Upon decomposition, organic manures release inorganic nutrients that stimulate plankton growth at the base trophic level of aquatic production cycle. Qualitative and quantitative analysis of plankton showed considerable differences in species diversity and abundance between culture systems fertilized with different organic manures (Nandeesha *et al.*, 1984; Kapur and Lal, 1986; Dhawan and Toor, 1989; Singh and Sharma, 1999). Differences in plankton concentration also varied when organic manure was applied at different rates (Yadava and Garg, 1992; Garg and Bhatnagar, 1996; Lin *et al.*, 1997; Azim *et al.*, 2001; Cheikyula *et al.*, 2001). However, Hickling (1962) and Hepher (1988) have demonstrated that above a certain level, increasing fertilizer rates do not further increase plankton concentration in the culture system.

2.7. Organic manuring and fish yields

Pekar and Olah (1990) reviewed the culture results of various food fish species in organically manured systems and found that the yield varied widely depending on the type of manure used, stocking rate, geo-climatic conditions and species cultured (**Table 4**). Lin *et al.* (1997) documented that yields of nile tilapia, *Oreochromis niloticus*, varied considerably in experimental ponds manured with different rates of poultry excreta and there was a significant correlation between fish yields and the available nitrogen in the pond system. However, yields of food fish cannot be compared with ornamental fish since the later are marketed as individuals and not on the basis of harvest weight, as is the case with food fish. Besides, the marketable size of ornamental fish varies considerably from food fish. According to our knowledge, at present, there is no documentation pertaining to culture and yields of ornamental fish species in organically manured culture systems under tropical conditions.

Table 4 Fish yields from manured ponds (after Pekar and Olah, 1990).

Type of manure	Fish stocked	Stocking rate (no./ ha)	Daily yield (kg/ ha)	Reference
Cattle manure	Common carp, Chinese carp and Tilapia	9000 – 18000	32	Moav *et al.* (1977)
	Tilapia	10000	16	Collis and Smitherman (1978)
Chicken manure	Common carp, Chinese carp and Tilapia	8000 – 16000	29 – 35	Wohlfarth *et al.* (1980)
	Common carp	2100	7	Bok and Jongblood (1984)
	Tilapia	10000	4.1 – 17.7	Teichert-Coddington and Green (1990)
Duck manure	Common carp, Silver carp and Tilapia	10000 – 20000	36	Barash *et al.* (1982)
Pig manure	Common carp and Chinese carp	10700	17 – 22	Buck *et al.* (1979)
	Common carp and Chinese carp	18000	40	Shan *et al.* (1985)
	Chinese carp and Tilapia	15500	36	Behrends *et al.* (1983)

2.8. Limitations of organic manuring

Pond fertilization through organic manuring is meant to boost fertility of pond water where nutrient concentrations are often too low to support desirable fish yields (Lin *et al.*, 1997). Fertilization also concomitantly affects water quality with positive or negative consequences to fish survival and growth. It is a well established fact that one of the most essential attributes towards the success of any aquaculture practice is the maintenance of appropriate water quality in the culture system (Diana *et al.*, 1997). However, pond fertilization using high amounts of organic manure can lead to reduced water quality including severe depletion of dissolved oxygen and generation of H_2S, methane and ammonia (Chattopadhyay and Mandal, 1980; Wong *et al.*, 1982; Batterson *et al.*, 1989; Green *et al.*, 1989; Boyd, 1990; Singh *et al.*, 1991), causing stress to impairment of normal metabolism in fish (Wong *et al.*, 1979), and even death of young fish larvae (Yip and Wong, 1977). Fish mortality has also been attributed to spikes in un-ionized ammonia in ponds receiving a combination of inorganic (urea) and organic fertilizers (poultry excreta) in high doses (Teichert-Coddington *et al.*, 1992). Besides, stress in fish due to reduced water quality can ultimately lead to exhaustion and disease (Francis-Floyd, 1990). Since ornamental fish, unlike food fish, are sold as individuals and have to be visually attractive to be accepted in the market, stressed or exhausted fish may be aesthetically unattractive to potential customers.

Primary production in excessively fertilized ponds can limit light penetration (Hepher, 1962). Pond fertilization using high amounts of animal wastes are known to have caused noticeable harm to the environment (Quines, 1988), by proliferating the growth of pathogenic bacteria like *Aeromonas* sp. and *Pseudomonas* sp. in the waterbody (Hojovec, 1977; Sugita *et al.*, 1985a; Jinyi *et al.*, 1987; Iger *et al.*, 1988; Okaeme and Olufemi, 1997). Also, organic manure are known to have caused *Aeromonas punctata* and *Saprolegina parasitica* epizootics in rohu, *Labeo rohita* and common carp (Toor *et al.*, 1983). Since ornamental fish are not consumed, public health may not be directly affected by ornamental fish cultured in manured systems. However, manures should be applied judiciously so that fish health is not negatively affected.

2.9. Some farmer-friendly aquaculture management techniques with emphasis to Indian conditions

Specific pond management techniques need to be developed to create the best environment for the fish, while utilizing animal wastes that can sustain productivity at low cost. Fertilization with manure with water exchange, have proved to be more effective than manured systems without water exchange, in maintaining better water quality and lower mortality rates in common carp (Chakrabarti and Jana, 1990), and Indian Major Carps, rohu and mrigal, *Cirrhinus mrigala* (Chakrabarti and Jana, 1998).

Introduction of live zooplankton has been investigated as an alternate to pond fertilization for increasing yields of several food fish species while avoiding water quality deterioration (Jana and Pal, 1987). However, as also mentioned earlier, literature on ornamental fish rearing under Indian conditions is scanty. As the present trend among ornamental fish producers is to utilize information generated from food fish aquaculture (Watson and Shireman, 1996), experiments on supply of exogenous zooplankton could be investigated with ornamental fish species.

3. Materials and Methods

3.1. Experimental design

With a view to determining the effect of different management regimes on the survival and growth of ornamental fish, koi carp (*Cyprinus carpio* L.), under tropical conditions, the present investigation was conducted in 9 phases (experiments) in both field and laboratory conditions.

3.1.1. Period of study

Nine experiments were conducted from February, 2002 to November, 2004. Fish culture experiments (field experiments) were generally of 3-month (Experiment Nos. 1, 2 and 3) or 11-week (Experiment Nos. 4, 5, 6, 7 and 9) duration. The laboratory-based behaviour experiment (Experiment No. 8) was carried out during 15 consecutive days (20 August to 3 September, 2004). More details on the period of study for any particular experiment are presented later, while elaborating the experimental procedures of each experiment.

3.1.2. Site of study and infrastructure

The field experiments were conducted in the Rainbow Ornamental Fish Farm and adjoining areas in Raninagar village, Jalpaiguri district, West Bengal, India. The laboratory analysis for all the field experiments and the behaviour experiment (Experiment No. 8) were conducted in the Aquaculture and Limnology Laboratory, Department of Zoology, University of North Bengal.

Two types of concrete tanks were used: small circular tanks (0.8 m diameter; capacity: 150 L) and large rectangular tanks (dimension 2.13 × 0.91 × 1.22 m; capacity: 2000 L). The ponds used in the experiments had a dimension of 9.14 × 6.10 × 1.07 m and a capacity of 59600 L. More details on the number of tanks/ponds used in any particular experiment and the aquaculture engineering/management techniques applied to the tanks/ponds during any particular experiment are presented later, while discussing the experimental procedures of each experiment.

3.1.3. Study animals

Koi carp (*Cyprinus carpio*), ornamental variety of the common carp were used in the experiments. In the final two experiments (relating to behaviour study and experimental polyculture of ornamental carp), goldfish (*Carassius auratus*) were also

used along with koi carp. All the experimental fish were procured from Rainbow Ornamental Fish Farm, Jalpaiguri, which in one of the leading ornamental fish hatcheries in India, and the largest in North Bengal region (also the site of our rearing experiments). Since ornamental fish breeding and culture is still in its initial stages in India, and there is a dearth of selected brooder fish, it was difficult to obtain any particular type of koi carp larvae for the experiments. Therefore, we had to depend on supplies that included the offspring of mixed commercial production by parents of different koi types, namely, Kohaku, Bekko, Showa and Asagi koi. More details on the number and type of koi carp or goldfish used in any particular experiment and the average size of fish procured are presented later, while discussing the experimental procedures of each experiment. The systematic position of koi carp and gold fish are detailed below.

3.1.3.1. Systematic position of koi carp

Phylum Chordata
Sub phylum Vertebrata
Super class Osteichthyes
Class Actinopterygii
Sub class Neopterygii
Infra class Teleostei
Super order Ostariophysi
Order Cypriniformes
Family Cyprinidae
Genus *Cyprinus*
Species *carpio*
Sub species *koi**

Scientific name: *Cyprinus carpio* L.

* Subspecies has not been assigned official Latin name yet.

3.1.3.2. Systematic position of goldfish

Phylum Chordata
Sub phylum Vertebrata
Super class Osteichthyes
Class Actinopterygii
Sub class Neopterygii
Infra class Teleostei
Super order Ostariophysi
Order Cypriniformes
Family Cyprinidae
Genus *Carassius*
Species *auratus*

Scientific name: *Carassius auratus* (L.)

3.2. Field experiments

3.2.1. Studies on the effect of manuring rate on fish production (Experiment No. 1)

3.2.1.1. Experimental procedure

The koi carp used in this study were the offspring of a mixed commercial production by 18 pairs of parents of Kohaku and Showa koi types. The experiment was conducted in 21 outdoor concrete tanks (capacity: 2000 L). A 3.175 cm GI pipe was fitted to the outlet of each tank in such a way, that water in excess of 2000 L would automatically flow out. A plankton net (No. 21 with 77 mesh/ cm^2) bordered the outlet preventing escape of plankton with the outflowing water. A 10 cm layer of soil was placed on the bottom of each tank, which was then filled with 2000 L of groundwater 10 days prior to stocking. This interval after manure application is a prerequisite for establishing satisfactory environmental conditions for optimum zooplankton production in tanks (Jana and Chakrabarti, 1993). After a one-week acclimatization period, 8400 koi carp larvae, 2 - 3 weeks old (0.09 ± 0.025 g), were equally distributed to each tank (400 fish/ tank). Since the stocking density was not standardized, fish were stocked at 0.2 fish/ L, commonly practiced in ornamental fish farms in Asia (Fernando and Phang, 1985). To study the effect of different application rates of cow and poultry manure on its survival and production, fish were treated for 90 days (March to May, 2002), with the seven treatments:

(1) cow dung applied at 0.13 kg/ m^3 every 10 days (C1);

(2) cow dung applied at 0.26 kg/ m^3 every 10 days (C2);

(3) cow dung applied at 0.39 kg/ m^3 every 10 days (C3);

(4) poultry manure applied at 0.13 kg/ m^3 every 10 days (P1);

(5) poultry manure applied at 0.26 kg/ m^3 every 10 days (P2);

(6) poultry manure applied at 0.39 kg/ m^3 every 10 days (P3); and

(7) a control treatment in which a commercial pelleted diet was used as feed (C).

The application rates of the manures correspond to 1300 - 3900 kg/ ha. The high organic load was used in view of the high manuring rate (initial dose of 10000 kg/ ha and subsequent application of 5000 kg/ ha), recommended for nursery ponds in India (Jhingran, 1991). Three tanks were randomly assigned for each treatment. A single layer of nylon bird netting was used to cover the tanks. Most fish farmers in India cannot afford expensive aeration or mechanized water treatment equipments, hence water exchange in tanks is generally considered as a cost effective measure for water quality management. Since the water exchange rate was not standardized, an amount of 100 L water was replaced in each tank two times a week.

The manures used in the experiment were collected from local dairy and poultry farms, and allowed to decompose for 10 days prior to application. No manure was added to the tanks in the control treatment, where a commercial diet (Tokyu Corp., Japan) was applied. The diet contained 32% crude protein, 4% crude fat, 5% crude fibre, 10% crude ash, 9% moisture and 31% nitrogen free extract, and was selected on the basis of widespread availability. This level of crude protein corresponds to the established requirement for juvenile cyprinids fed to satiation (Lochmann and Phillips, 1994). The diet was applied at 5% body weight of stocked fish, daily, and was mechanically crumbled before being administered during the first month. For the rest of the study, original floating pellets (0.24 cm in diameter) were applied. Dry food was not applied to any other treatment, where the fish fed on naturally grown food.

3.2.1.2. Data collection

The amount of total nitrogen and organic carbon in the manures was estimated according to methods described later. Water samples were collected weekly from each tank and transported to the laboratory within 2 hours. Routine water quality parameters were estimated according to methods described later. Samples of plankton were collected with plankton net, which was made of standard bolting silk cloth (No. 21 with 77 mesh/ cm^2) two times a week and analyzed according to methods described later.

Fish were harvested after 90 days and weighed. Individual weight gain, Specific Growth Rate (SGR) and survival rate were calculated for each treatment as described later. Results in percentage were normalized using Arc Sin Angular Transformation

Method (ASATM) (Mosteller and Youtz, 1961; Sokal and Rohlf, 1969) before being subjected to further statistical analysis.

3.2.1.3. Statistical analyses

A one-way analysis of variance (ANOVA) procedure was performed to detect significant differences in water quality parameters as well as growth, survival rate and SGR among treatments. A Tukey's Honestly Significant Difference (THSD) test (Zar, 1999) was used to compare and rank means. A level of significance of $P < 0.05$ was used. The degree of linear relationship between plankton density and fish growth was determined by means of correlation coefficients following Karl Pearson's method (Sunder Rao and Richard, 1999).

tank and transported to the laboratory within 2 hours. Routine water quality parameters were estimated according to methods described later. Samples of plankton were collected with plankton net, which was made of standard bolting silk cloth (No. 21 with 77 mesh/ cm^2) two times a week and analyzed according to methods described later.

Fish were harvested after 90 days and weighed. Individual weight gain, SGR, survival rate and the number of marketable fish were calculated for each treatment as described later. Results in percentage were normalized using ASATM (Mosteller and Youtz, 1961; Sokal and Rohlf, 1969) before being subjected to further statistical analysis.

3.2.2.3. Statistical analyses

A one-way ANOVA procedure was performed to detect significant differences in water quality parameters as well as growth, SGR, survival rate and number of marketable fish among treatments. A THSD test (Zar, 1999) was used to compare and rank means. A level of significance of $P < 0.05$ was used. The degree of linear relationship between plankton density and fish growth was determined by means of correlation coefficients following Karl Pearson's method (Sunder Rao and Richard, 1999).

3.2.3. Studies on the effect of stocking density on fish production (Experiment No. 3)

3.2.3.1. Experimental procedure

The koi carp used in this study were the offspring of a mixed commercial production by 25 pairs of parents of Kohaku, Asagi, and Bekko koi types. The experiment was conducted in fifteen circular concrete tanks (capacity: 150 L). A 3.175 cm GI pipe was fitted to the outlet of each tank in such a way, that water in excess of 150 L would automatically flow out. Fish were subjected to one-week acclimatization period prior to the experiment. To study the effect of stocking density on its survival and production, 675 koi carp larvae, about 3 weeks old (0.14 ± 0.035 g), were randomly assigned to the tanks and were cultured for 90 days (September to November, 2002) at the following densities:

(1) 0.1 fish/ L (D1);

(2) 0.2 fish/ L (D2);

(3) 0.3 fish/ L (D3);

(4) 0.4 fish/ L (D4); and

(5) 0.5 fish/ L (D5).

Three tanks were randomly assigned for each treatment. A single layer of nylon bird netting was used to cover the tanks. All fish were fed three times a day during the 90-day culture period, slightly in excess of satiation to eliminate the possibility that insufficient food supply may influence growth. The fish were given a commercial pelleted diet (Tokyu Corp., Japan) at the amount of 5% body weight of stocked fish daily. For water quality management, 5% of water was replaced in each tank every day.

3.2.3.2. Data collection

Water samples were collected weekly from each tank and transported to the laboratory within 2 hours. Routine water quality parameters were estimated according to methods described later. Fish were harvested after 90 days and weighed. Individual weight gain, SGR, Food Conversion Ratio (FCR), survival rate and the number of

marketable fish were calculated for each treatment as described later. Results in percentage were normalized using ASATM (Mosteller and Youtz, 1961; Sokal and Rohlf, 1969) before being subjected to further statistical analysis.

3.2.3.3. Statistical analyses

A one-way ANOVA procedure was performed to detect significant differences in water quality parameters as well as growth, SGR, FCR, survival rate and number of marketable fish among treatments. A THSD test (Zar, 1999) was used to compare and rank means. A level of significance of $P < 0.05$ was used.

3.2.4. Studies on the effect of live-food treatment on fish production against conventional manuring regimen. (A) Comparative account of fish production throughout different seasons (culture periods) in a year, namely, winter, summer, monsoon and post monsoon (Experiment No. 4)

3.2.4.1. Experimental procedure

In Jalpaiguri district of North Bengal, four prominent seasons are distinguished in a year. Our experiments constituted four 11-week growth trials, that were conducted throughout one year, with one trial being conducted in every season: (1) 4 December, 2002 to 19 February, 2003 (Winter); (2) 4 March, 2003 to 20 May, 2003 (Summer); (3) 3 June, 2003 to 19 August, 2003 (Monsoon); and (4) 2 September, 2003 to 18 November, 2003 (Post Monsoon).

The koi carp used in this study were the offspring of mixed commercial production by 50 pairs of parents of Kohaku, Asagi, Bekko and Showa koi types. Each seasonal experiment were conducted in 12 outdoor concrete tanks (capacity: 2000 L). A 3.175 cm GI pipe was fitted to the outlet of each tank in such a way, that water in excess of 2000 L would automatically flow out. A plankton net (No. 21 with 77 mesh/ cm^2) bordered the outlet preventing escape of plankton with the outflowing water. A 10 cm layer of soil was placed on the bottom of each tank, which was then filled with 2000 L of groundwater 10 days prior to stocking. To study the effect of live-food treatment on fish production against conventional manuring regimen, fish were reared for 11 weeks according to one of the four treatment regimes:

(1) introduction of live zooplankton (live-food system or LF);

(2) direct fertilization with poultry manure (PM);

(3) direct fertilization with cow dung (CD); and

(4) introduction of a commercial pelleted diet (Tokyu Corp., Japan) into the tanks (control system or C).

Three tanks were randomly assigned for each treatment. The poultry manure and cow dung were both applied at a dose of 0.26 kg/ m^3, every 10 days, in the PM and CD treatments, respectively, as standardized in Experiment No. 1. For water quality

management, every day about 5% water (100 L) was replaced in each tank, as standardized in Experiment No. 2. The stocking density was maintained at 600 fish/ tank or 0.3 fish/ L, as optimized in Experiment No. 3. The fish in each LF tank were fed by transferring about 60 L of plankton-rich water every day from a series of plankton culture tanks that were fertilized with poultry manure and maintained under similar management conditions as the PM treatment. About 60 L of excess water was discharged every day from the LF tanks, during the introduction of plankton-rich water. A single layer of nylon bird netting was used to cover the tanks. Constant water levels were maintained in the culture tanks by supplying groundwater to compensate for loss due to evaporation.

3.2.4.2. Data collection

The amount of total nitrogen and organic carbon in the manures was estimated according to methods described later. In each seasonal trial, water samples were collected weekly from each tank and transported to the laboratory within 2 hours. Routine water quality parameters were estimated according to methods described later. Samples of plankton were collected with plankton net, which was made of standard bolting silk cloth (No. 21 with 77 mesh/ cm^2) two times a week and analyzed according to methods described later.

In each seasonal trial, fish were harvested after 11 weeks and weighed. Individual weight gain, SGR, survival rate, number of fish with deformities and the number of marketable fish were calculated for each treatment as described later. Results in percentage were normalized using ASATM (Mosteller and Youtz, 1961; Sokal and Rohlf, 1969) before being subjected to further statistical analysis.

3.2.4.3. Statistical analyses

In each seasonal trial, a one-way ANOVA procedure was performed to detect significant differences in water quality parameters as well as growth, SGR, survival rate, number of deformed fish and number of marketable fish among treatments. A THSD test (Zar, 1999) was used to compare and rank means. A level of significance of $P < 0.05$ was used. The degree of linear relationship between plankton density and fish growth was determined by means of correlation coefficients following Karl Pearson's method (Sunder Rao and Richard, 1999).

3.2.5. Studies on the effect of live-food treatment on fish production against conventional manuring regimen. (B) Comparative account of fish production in different culture systems, namely, concrete tanks and earthen ponds (Experiment No. 5)

3.2.5.1. Experimental procedure

The koi carp used in this study were the offspring of a mixed commercial production by 50 pairs of Asagi, Bekko, Kohaku and Showa koi types. The experiment was conducted in 12 concrete tanks (capacity: 2000 L each) and 12 earthen ponds (capacity: 59600 L each). A 3.175 cm GI pipe was fitted to the outlet of each tank in such a way, that water in excess of 2000 L would automatically flow out. A 10 cm layer of soil was placed on the bottom of each tank, which was then filled with 2000 L of groundwater 10 days prior to stocking. The preparation of the ponds for the experiment started in September, 2003 when they were emptied of water and sun dried for 2 weeks. Late rainfall during October, 2003 filled the ponds.

After a one-week acclimatization period, koi carp larvae, 2 - 3 weeks old (0.12 ± 0.008 g), were randomly assigned to the culture tanks and ponds, and were reared for 11 weeks (2 December, 2003 to 17 February, 2004) according to one of the four management regimes for each of the tank and pond culture systems:

(1) introduction of exogenous plankton into the culture tanks (TLF) and ponds (PLF). Live plankton was cultured in a series of plankton culture ponds where poultry manure was applied as fertilizer every 10 days at 0.26 kg/ m^3, as standardized in Experiment No. 1. For feeding the larvae, about 30 L and 1000 L of plankton-rich water, respectively, was transferred to each fish culture tank and pond;

(2) direct application of poultry manure in the tanks (TPM) and ponds (PPM) at a dose as mentioned above;

(3) direct application of cow dung in the tanks (TCD) and ponds (PCD) at the similar dose, as mentioned above; and

(4) a control treatment for the tanks (TC) and ponds (PC), where an imported pelleted diet (Tokyu Corp., Japan) was applied.

There were three replicates for each treatment. The entire experimental unit was covered by a single layer of nylon bird netting. Fish were maintained at a stocking density of 0.3 fish/ L, as optimized in Experiment No. 3. For water quality management, every day about 5% water (100 L) was replaced in each tank, as standardized in Experiment No. 2. However, there was no water exchange for the ponds. Constant water levels were maintained in the culture tanks and ponds by supplying ground water periodically to compensate for loss due to evaporation. Approximately 30 L and 1000 L of excess water was discharged from the live-food tanks (TLF) and ponds (PLF), respectively, every day during the introduction of plankton-rich water. A plankton net (No. 21 with 77 mesh/ cm^2) bordered the outlet preventing escape of plankton with the outflowing water.

3.2.5.2. Data collection

The amount of total nitrogen and organic carbon in the manures was estimated according to methods described later. Water samples were collected weekly from the tanks and ponds and transported to the laboratory within 2 hours. Routine water quality parameters were estimated according to methods described later. Samples of plankton were collected with plankton net, which was made of standard bolting silk cloth (No. 21 with 77 mesh/ cm^2) two times a week and analyzed according to methods described later.

Fish were harvested after 11 weeks and weighed. Individual weight gain, SGR, survival rate, number of fish with deformities and the number of marketable fish were calculated for each treatment as described later. During the process, all the fish stocked in the tanks were weighed individually. However, for the four treatments maintained in ponds, individual data could not be recorded from every harvested fish. In its place, 1000 fish were randomly selected from each unit so as to represent the entire pond, and data relating to fish growth parameters were collected from these fishes. Results in percentage were normalized using ASATM (Mosteller and Youtz, 1961; Sokal and Rohlf, 1969) before being subjected to further statistical analysis.

3.2.5.3. Statistical analyses

A one-way ANOVA procedure was performed to detect significant differences in water quality parameters as well as growth, SGR, survival rate, number of deformed fish and number of marketable fish among treatments. A THSD test (Zar, 1999) was used to compare and rank means. A level of significance of $P < 0.05$ was used.

3.2.6. Studies on effect of live-food treatment on fish production against conventional manuring regimen. (C) Examination of food selection and food preference of cultured fish in the different treatments (Experiment No. 6)

3.2.6.1. Experimental procedure

The koi carp used in this study were the offspring of a mixed commercial production by 40 pairs of Asagi, Bekko and Showa koi types. After a one-week acclimatization period, koi carp larvae, 2 - 3 weeks old (0.13 ± 0.015 g), were divided into two batches for the experiment.

The first batch was reared in 12 earthen ponds (capacity: 59600 L) and used for the growth studies. Larvae were stocked at a density of 0.3 fish/ L, as optimized in Experiment No. 3. Fish were reared for 11 weeks (3 March to 19 May, 2004) according to one of the four treatment regimes:

(1) live-food ponds (LF), into which about 1000 L of plankton-rich water was transferred every day from a series of ponds culturing live plankton. The ponds used for culturing plankton were fertilized with poultry manure using a dose of 0.26 kg/ m^3 every 10 days, as standardized in Experiment No. 1;

(2) poultry manure ponds (PM), where poultry excreta was added directly as manure to the ponds at the above dose;

(3) cow dung ponds (CD), where cow dung was applied as manure to the ponds at the above dose; and

(4) control ponds (C), in which a commercial pelleted diet (Tokyu Corp., Japan) was used.

Three ponds were randomly assigned for each treatment. Constant water levels were maintained in the culture ponds by supplying ground water to compensate for loss due to evaporation. In the live-food system (LF), about 1000 L of excess water would be discharged every day during the introduction of plankton-rich water. A plankton net (No. 21 with 77 mesh/ cm^2) bordered the outlet preventing escape of plankton with the outflowing water. The entire experimental unit was covered by a single layer of nylon bird netting.

The second batch was used for studying the food selection. A 10 cm layer of soil was placed on the bottom of six outdoor concrete tanks (capacity: 2000 L), which was then filled with control pond water. The tanks were fertilized with poultry manure at the rate of 0.26 kg/ m^3 every 10 days. Fish were stocked and maintained at a density of 0.3 fish/ L. Forty eight fish were removed randomly at weekly intervals from the outdoor tanks. Each fry was placed in a plastic container (5 L capacity) and starved for 48 hours for gastric evacuation under laboratory conditions. Four containers, each containing one fry, were randomly placed on the bottom of different areas of each of the above 12 ponds (three for each management regime). The containers were covered with a net to prevent fish escaping but still permit free movement of plankton between the jar and the surrounding water. As such, the containers represented the management regime of the pond in which they were placed, in terms of water quality and plankton diversity. After 12 hours, the fish were removed from the containers and sacrificed for examination of food selection and food consumption.

3.2.6.2. Data collection

The amount of total nitrogen and organic carbon in the manures was estimated according to methods described later. Water samples were collected weekly from the ponds and transported to the laboratory within 2 hours. Routine water quality parameters were estimated according to methods described later. Samples of plankton were collected with plankton net, which was made of standard bolting silk cloth (No. 21 with 77 mesh/ cm^2) two times a week and analyzed according to methods described later.

Fish were harvested after 11 weeks and weighed. Individual weight gain, SGR, survival rate, number of fish with deformities and the number of marketable fish were calculated for each treatment as described later. However, individual data could not be recorded from every harvested fish. In its place, 1000 fish were randomly selected from each unit so as to represent the entire pond, and data relating to fish growth parameters were collected from these fishes.

Qualitative and quantitative estimation of plankton consumed was done by analyzing the gut of koi carp of the second batch. Routine examination procedures were followed for gut analysis (Jhingran *et al.*, 1988). Average values from 12 containers used for each regime were used for further calculations. The extent of prey selection by koi carp was determined according to methods as described later. Results in percentage were normalized using ASATM (Mosteller and Youtz, 1961; Sokal and Rohlf, 1969) before being subjected to further statistical analysis.

3.2.6.3. Statistical analyses

A one-way ANOVA procedure was performed to detect significant differences in water quality parameters as well as growth, SGR, survival rate, number of deformed fish and number of marketable fish among treatments. A THSD test (Zar, 1999) was used to compare and rank means. A level of significance of $P < 0.05$ was used.

3.2.7. Studies on the effect of live-food treatment on fish production against conventional manuring regimen. (D) Estimation of bacteriological counts of water and bottom sediment in the different treatments (Experiment No. 7)

3.2.7.1. Experimental procedure

The koi carp used in this study were the offspring of a mixed commercial production by 40 pairs of Asagi, Bekko, Kohaku and Showa koi types. The experiment was conducted in 12 earthen ponds (capacity: 59600 L each). After a one-week acclimatization period, koi carp larvae, 2-3 weeks old (0.12 ± 0.007 g), were stocked in the experimental ponds and maintained at a density of 0.3 fish/ L, as optimized in Experiment No. 3. Fish were reared for 11 weeks (02 June to 18 August, 2004) according to one of the four treatment regimes:

(1) live-food ponds (LF), into which about 1000 L of plankton-rich water was transferred every day from a series of ponds culturing live plankton. The ponds used for culturing plankton were fertilized with poultry manure using a dose of 0.26 kg/ m^3 every 10 days, as standardized in Experiment No. 1;

(2) poultry manure ponds (PM), where poultry excreta was added directly as manure to the ponds at the above dose;

(3) cow dung ponds (CD), where cow dung was applied as manure to the ponds at the above dose; and

(4) control ponds (C), in which a commercial pelleted diet (Tokyu Corp., Japan) was used.

Three ponds were randomly assigned for each treatment. A single layer of nylon bird netting was used to cover the ponds. Constant water levels were maintained in the experimental ponds by supplying ground water periodically to compensate for loss due to evaporation. However, as the experiment was conducted during the rainy season, evaporation losses were minimal and were mostly compensated naturally, by rainwater. Approximately 1000 L of excess water was discharged from the live food ponds (LF), everyday during the introduction of plankton-rich water. A plankton net (No. 21 with 77 mesh/ cm^2) bordered the outlet preventing escape of plankton with the outflowing water.

3.2.7.2. Data collection

The amount of total nitrogen and organic carbon in the manures and sediment samples were estimated according to methods described later. Water and sediment samples were collected weekly from the ponds and transported to the laboratory within 2 hours. Routine physico-chemical parameters were estimated according to methods described later. Samples of plankton were collected with plankton net, which was made of standard bolting silk cloth (No. 21 with 77 mesh/ cm^2) two times a week and analyzed according to methods described later.

For bacteriological analysis, water samples were collected weekly in presterilized glass bottles (125 mL), and processed within 6 hours of collection. Weekly sediment samples were collected using hand and stored in presterilized plastic containers. Isolation and enumerations of total aerobic heterotrophic bacteria, *Aeromonas* sp. and *Pseudomonas* sp. were carried out according to methods described later.

Fish were harvested after 11 weeks and weighed. Individual weight gain, SGR, survival rate, number of fish with deformities and the number of marketable fish were calculated for each treatment as described later. However, individual data could not be recorded from every harvested fish. In its place, 1000 fish were randomly selected from each unit so as to represent the entire pond, and data relating to fish growth parameters were collected from these fishes. Results in percentage were normalized using ASATM (Mosteller and Youtz, 1961; Sokal and Rohlf, 1969) before being subjected to further statistical analysis.

3.2.7.3. Statistical analyses

A one-way ANOVA procedure was performed to detect significant differences in water quality parameters as well as growth, SGR, survival rate, number of deformed fish and number of marketable fish among treatments. A THSD test (Zar, 1999) was used to compare and rank means. A level of significance of $P < 0.05$ was used.

3.2.8. Studies on experimental ornamental fish polyculture. (A) Behavioural responses of two popular ornamental carps, koi carp and goldfish to monoculture and polyculture conditions in aquaria (Experiment No. 8)

3.2.8.1. Experimental procedure and data collection

About 2 - 3 weeks old larvae of koi carp (0.13 ± 0.03 g), and goldfish (0.18 ± 0.05 g) were brought to the laboratory, where they were acclimated for nearly 3 weeks prior to study. The koi carp used in this study were the offspring of five pairs of parents of Bekko koi type, while the goldfish were of normal (common) variety. At first, the koi carp and goldfish larvae were divided into two batches. Both batches of fish were treated to similar acclimation and experimental protocols, except for the food applied to each batch, which was different. Fish were stocked in filtered and aerated single species aquaria and maintained under artificial lights (12 hours light: 12 hours dark photoperiod). The tanks were filled with tap water (water volume 150 L; 20 fish/ tank). The physico-chemical parameters were similar in all the holding tanks (pH 7.0 - 7.2; dissolved oxygen 6.5 - 6.8 mg/ L and water temperature 24^{0}C - 26^{0}C). Fish were fed with live tubifex worm (first batch) and live zooplankton (second batch) during this period in the amount of 5% body weight of stocked fish daily (adjusted every weekly). The same food was used later during the experiments. The tubifex worm was procured from the local market, while zooplankton was cultured in outdoor concrete tanks (capacity: 2000 L) under management conditions similar to the live plankton culture tanks in Experiment No. 4.

Two parallel experiments were conducted for the two batches during 15 consecutive days from 20 August to 3 September, 2004 in six 150 L tanks (three tanks for each experiment) maintained under similar physico-chemical conditions as the holding tanks (pH 6.9 - 7.2; dissolved oxygen 6.5 - 6.9 mg/ L and water temperature 23^{0}C - 26^{0}C). In these trials, monoculture or polyculture groups totaling 20 fish were used in each aquarium. The treatments (for each batch) were:

(1) koi carp, monoculture;

(2) goldfish, monoculture; and

(3) koi carp and goldfish, polyculture, stocked at 1:1.

On an average, the 5 - 6 weeks old fish used during the study had a mean weight of 0.86 ± 0.11 g (koi carp) and 1.49 ± 0.38 g (goldfish). The order of trials was randomized with respect to treatment and 15 replicate trials (one trial every day) were performed for each treatment. Grid markings on the tanks allowed the vertical height in the water column of the fish to be estimated. The tanks were screened with black art paper from four sides and an eye-slit was cut on the front side allowing the experimenter to observe and record the behaviour of fish without disturbing them. In each trial, the fish were placed in the tank and left to settle for 1 hour. This was followed by a period of focal sampling (Martin and Bateson, 1990) to record the behaviour of individual fish: a randomly selected individual for each species was watched for 15 minutes, during which time, its depth was recorded every 30 seconds by reference to the markings on the tank. This was followed by a 5-minute sampling period in which the occurrence and direction of all agonistic interactions (chases, nips) involving a randomly chosen individual of each species were recorded. Then a small amount of food was applied and the above procedures, involving both periods of focal sampling were repeated. Each trial therefore yielded data on aggressive encounters and the depth of the species concerned, both in presence and absence of food.

Aggressive behaviour was measured as the number of attacks, defined as accelerated swimming movements (chases), followed by nips - a bite, and/or flight or pursuit. In the first batch, live tubifex worm was applied in feeding baskets and placed on the floor of the tanks. For the second batch, the food (plankton) was added centrally onto the water surface and the live plankton dispersed rapidly over the entire water surface. This way of offering food generally results in greater feeding opportunity and promotes uniformity of feeding and growth (McCarthy *et al.*, 1999; Gomez-Laplaza, 2002).

3.2.8.2. Statistical analyses

Differences in the mean chasing frequency, mean nipping frequency and mean depth recorded for each species between the monoculture and polyculture treatments were examined using Student's paired t-test (Gupta, 2000). Significance was accepted at $P < 0.05$. Separate analyses were carried out on the 'food present' and 'food absent'

data for each batch. Primary data (number of nips and chases) were log-transformed prior to analysis and standardized by dividing the number of attacks received by focal fish per trial by the number of potential aggressors. For interspecific attacks, the number of aggressors was 'n', where n = group size of the attacking species. For conspecific attacks, the number of aggressors was n - 1.

3.2.9. Studies on experimental ornamental fish polyculture. (B) Comparison of food selection and growth performance of koi carp and goldfish in monoculture and polyculture rearing in tropical ponds (Experiment No. 9)

3.2.9.1. Experimental procedure

The koi carp used in this study were the offspring of a mixed commercial production by 40 pairs of Asagi, Bekko and Showa koi types, while the goldfish were of normal (common) variety. After a one-week acclimatization period, 2 - 3 weeks old larvae of koi carp (0.12 ± 0.014 g) and goldfish (0.16 ± 0.018 g) were divided into two batches for the experiment.

The first batch of fish was reared for 11 weeks (5 September to 18 November, 2004) in 21 earthen ponds (capacity: 59,600 L) in Raninagar village, Jalpaiguri, India. To study their growth, survival and food selection under monoculture and different polyculture regimes, seven treatments were examined:

(1) koi carp monoculture (100%K);

(2) 90% koi carp and 10% goldfish (90%K-10%G);

(3) 70% koi carp and 30% goldfish (70%K-30%G);

(4) 50% koi carp and 50% goldfish (50%K-50%G);

(5) 30% koi carp and 70% goldfish (30%K-70%G);

(6) 10% koi carp and 90% goldfish (10%K-90%G); and

(7) goldfish monoculture (100%G).

There were three replicates for each treatment. Fish were maintained at a stocking density of 0.3 fish/ L, as optimized in Experiment No. 3. Fish in each pond were fed by transferring about 1000 L of plankton-rich water from a series of ponds culturing plankton. The ponds used for culturing plankton were fertilized with poultry manure using a dose of 0.26 kg/ m^3 every 10 days, as standardized in Experiment No. 1. During the introduction of plankton-rich water, about 1000 L of excess water was discharged everyday from each pond. A plankton net (No. 21 with 77 mesh/ cm^2) bordered the outlet preventing escape of plankton with the outflowing water.

Every week, four fish of each species were randomly removed from each pond and sacrificed for examination of food selection and food consumption. The second batch of fish were maintained at a stocking density of 0.3 fish/ L in seven outdoor concrete tanks (capacity: 2000 L) under seven treatments of monoculture and polyculture combinations, similar to the first batch. About 30 L of plankton water were channeled into each tank daily for feeding the fish and similar amount of excess water was discharged. Fish cultured in these seven tanks were transferred to the experimental ponds corresponding to their respective treatments from time to time to substitute the fish of the first batch, which were captured for gut examination every weekly, to ensure that the results of the growth trial were not affected.

3.2.9.2. Data collection

Water samples were collected weekly from the ponds and transported to the laboratory within 2 hours. Routine water quality parameters were estimated according to methods described later. Samples of plankton were collected with plankton net, which was made of standard bolting silk cloth (No. 21 with 77 mesh/ cm^2) two times a week and analyzed according to methods described later.

Fish were harvested after 11 weeks and weighed. Individual weight gain, SGR, survival rate, number of fish with deformities and the number of marketable fish for each species were calculated for each treatment as described later. However, individual data could not be recorded from every harvested fish. In its place, 500 fish of each species were randomly selected from each unit so as to represent the entire pond, and data relating to fish growth parameters were collected from these fishes.

Qualitative and quantitative estimation of plankton consumed was done by analyzing the gut of koi carp and goldfish every weekly. Routine examination procedures were followed for gut analysis (Jhingran *et al.*, 1988). The extent of prey selection by koi carp was determined according to methods as described later. Results in percentage were normalized using ASATM (Mosteller and Youtz, 1961; Sokal and Rohlf, 1969) before being subjected to further statistical analysis.

3.2.9.3. Statistical analyses

A one-way ANOVA procedure was performed to detect significant differences in water quality parameters as well as growth, SGR, survival rate and number of deformed fish for each species among treatments. The total number of marketable fish (koi carp plus goldfish) produced in each treatment was also assessed by one-way ANOVA. A THSD test (Zar, 1999) was used to compare and rank means. A level of significance of $P < 0.05$ was used.

3.3. Methodology

3.3.1. Water quality analyses

Routine water quality parameters like Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Free Carbon dioxide (Free CO_2), Carbonate (CO_3) and Bicarbonate (HCO_3) alkalinity were determined titrametrically according to methods as described by APHA (1998). Specific conductivity was determined using a conductivity meter bridge (Systronics, Model 304). Temperature was recorded by a mercury thermometer. The pH was measured using a portable pH meter (Hanna Instruments, Model pHep).

With the aid of spectrophotometer (Systronics, Model 106), the concentration of nutrients like Ammonium - N (NH_4 - N; Phenol-hypochlorite Method), Nitrite - N (NO_2 - N; α-Napthalamine and Sulphanilic Acid Method) and Phosphate - P (PO_4 - P; Stannous-Chloride Method) was determined according to methods as described by APHA (1998). The concentration of Nitrate - N (NO_3 - N; Brucine Method) was also determined using a spectrophotometer (Systronics, Model 106) as described by Trivedy and Goel (1984).

3.3.2. Estimation of Total Nitrogen and Organic Carbon in the manure and sediment samples

The amount of Total Nitrogen and Organic Carbon in the organic manures in all the field experiments and sediment samples (Experiment No. 7) was estimated according to Micro-Kjeldahl's Method (Anderson and Ingram, 1993) and Wet Oxidation Method (Walkley and Black, 1934), respectively.

3.3.3. Plankton analyses

Samples of plankton were collected with plankton net, which was made of standard bolting silk cloth (No. 21 with 77 mesh/ cm^2). Collected plankton were concentrated to 20 mL, and preserved in 4% formalin. Enumerations of 1 mL of concentrated plankton were performed under the microscope using Sedgwick Rafter Counting Cell according to methods as described by APHA (1998). Plankton was identified with the aid of Needham and Needham (1963), and Battish (1992).

3.3.4. Analyses of fish growth parameters

Fish were weighed both at the beginning of each experiment and at harvest. For recording the weight, fish were captured and excess water removed on paper towel through the net before they were individually weighed to the nearest 0.001g. The selected fish were anaesthetized with Tricaine Methane Sulphonate (MS-222) of 0.04 g/ L concentration. The Specific Growth Rate (SGR) was calculated using the formula of Ricker (1975):

$$\mathbf{SGR = 100\ [(\ln W_t - \ln W_0)/t]}$$

where,

W_0 = initial live weight of the fish (g),

W_t = final live weight of the fish (g), and

T = culture period in days.

In Experiment No. 3, Food Conversion Rates (FCR) were expressed as Olivier and Kaiser (1997):

FCR = Diet fed (g)/ Weight gain (g) of the fish

Dead fish were removed daily, they were not replaced during the course of study, and differences between the number of fish stocked and the number of fish at harvest were used to calculate percent mortality in each treatment. In Experiment Nos. 4, 5, 6, 7 and 9, the number and percent of fish with body deformities were also recorded at harvest. However, fish deformities could not be recoded in the earlier experiments due to logistic reasons.

The number of marketable fish at the end of growth period in each experiment was calculated using the function for a normal distribution curve, where $z = (y - \mu)/ \sigma$; y is the least marketable weight (g), μ is the mean weight of the population, σ is the standard deviation of the total weight and z follows the standard normal probability distribution which determines the probability of finding fish above a given range. The number of marketable fish (n) was then determined using the table value of the normal probability distribution (P) as follows:

$$\mathbf{n = (1\text{-}P) \times F^*}$$

* For Experiment Nos. 2 and 3, F = total number of fish produced. For Experiment Nos. 4, 5, 6, 7 and 9, F = total number of fish produced excluding deformed individuals. (Fish deformities were not calculated for Experiment Nos. 2 and 3).

Marketable fish was not estimated for Experiment Nos. 1 and 8.

3.3.5. Estimation of food selection by fish

Gut content analyses were carried out for koi carp (Experiment Nos. 6 and 9) and goldfish (Experiment No. 9). The fish were dissected. The alimentary canal was removed without rupture or content-loss and weighed. Gut contents were preserved in 4% formalin. The weight of the gut content was determined as the difference between the weight of the full alimentary canal and the weight of the empty alimentary canal. The gut content was microscopically inspected for plankton and other available food particles. Availability of any ingredient in the gut was expressed as percent of that particular ingredient in gut contents:

Availability of any ingredient in the gut = [(Ingredient weight/ Gut content weight) × 100].

The extent of prey selection by fish was determined using Ivlev's formula (Ivlev, 1961):

$$E = (r_1 - p_1) / (r_1 + p_1)$$

where,

E = electivity value,

r_1 = relative quantity of any ingredient in the gut expressed as percent, and

p_1 = relative quantity of the same ingredient in the food complex, also expressed as percent.

The application of this formula gives a range of values from +1.0 for a very high degree of selection to -1.0 for a complete avoidance. A value of '0' indicates that the element is present in the diet in the same proportion as it is found in the environment, viz. complete absence of food selection.

3.3.6. Enumeration of bacterial population

In Experiment No. 7, water samples were collected in presterilized glass bottles (125 mL), while sediment samples were collected using hand and stored in presterilized plastic containers. All bottles and containers used in the bacteriological investigations were sterilized earlier by means of autoclaving at 15 lbs pressure (121^0 C) for 15 minutes. The suspension of sediment was prepared by mixing 1 g of wet sediment in 99 mL sterile distilled water. The Total Aerobic Heterotrophic Bacteria were enumerated in

Nutrient Agar Medium by serial dilution of the sample, followed by the conventional Spread Plate Technique (Chen and Kueh, 1976; Cappuccino and Sherman, 1992). *Aeromonas* sp. and *Pseudomonas* sp. were similarly enumerated on Aeromonas Isolation Medium Base and Pseudomonas Isolation Agar, respectively. All the bacteriological media were obtained from Himedia Laboratories Ltd., Mumbai, India. The compositions of the culture media are presented in Annexure 1. After inoculation, the petridishes containing the culture media were incubated at 37^0 C ± 1^0 C for 48 hours. The populations of bacteria were expressed in terms of cfus./ mL (colony forming units) in case of water, and cfus./ g for the sediments. Arithmetical means from three petridishes for each dilution were used in the study.

3.3.7. Statistical analyses

For verification of the recorded data in the different experiments, various statistical analysis were performed, namely, one-way ANOVA, THSD test, Student's paired t-test, Karl Pearson's correlation, Standard Deviation and Standard Error, Normal Distribution and so on. Details on the type of statistical analysis applied to any particular experiment have been elaborated earlier, while discussing the field experiments.

4. Results

4.1. Studies on the effect of manuring rate on fish production (Experiment No. 1)

4.1.1. Organic manure quality

The amount of total nitrogen in the cow and poultry manures was 2.15% and 2.66%, respectively, whereas, the amount of organic carbon was 21.24% and 30.19%, respectively.

4.1.2. Water quality

Water temperature was between 25°C and 31°C during the 90 days. However, there was no difference in the water temperature between the different treatments on any given sampling day. The pH ranged from 6.6 to 8.8 (**Table 5**). The DO never dropped below 5.0 mg/ L during the period of the study in any treatment except C3 and P3. Significantly higher values of PO_4 - P and specific conductivity were obtained in P3, compared to the other treatments ($P < 0.05$). CO_3 alkalinity was observed only in the treatments manured with poultry excreta (P1, P2, and P3), for very limited periods, when free CO_2 in these treatments was absent. The C3 and P3 treatments yielded significantly higher values ($P < 0.05$) of HCO_3 alkalinity, NH_4 - N, NO_3 - N and NO_2 - N, compared to the C1, P1 and control (C) treatments (**Table 5**).

4.1.3. Plankton abundance

Examination of plankton showed that the species diversity differed considerably between the manured treatments and control. Cladocerans, which formed a substantial proportion of the total plankton composition in the manured treatments, were absent from the control treatment. Other zooplankton and phytoplankton were represented in the control treatment with low numbers, compared with the manured treatments (**Table 6**). Within the manured treatments, the abundance (no./ L) of the different species varied considerably. Average zooplankton abundance was highest in P2, followed by C2, P3, C3, P1, C1 and C. The differences between treatments were significant ($P < 0.05$). Copepoda was the most dominant group, ranging from 52.5% of the total plankton composition in P2 to 88.74% in the control. The phytoplankton population in C3 and P3 was significantly higher ($P < 0.05$) than the other treatments (**Table 6**).

4.1.4. Fish growth and survival

At harvest, maximum weight gain of koi carp (**Table 7**) was achieved in the P2 treatment, followed in the decreasing order by the C2, P3, C3, P1, C1 and C treatments ($P < 0.05$). The SGR was quite high (> 3.5) in all treatments, though the differences among the various treatments were significant ($P < 0.05$). There was a significant difference ($P < 0.05$) in the survival of koi carp among the treatments, ranging from 65.5% in C to 86% in P2 (**Table 7**).

Table 5 Mean ± SE of major water quality parameters analyzed for the seven treatments. Each mean value represents 13 samples collected at weekly intervals during the 3-month growth period (March – May, 2002). Different superscripts in the same row indicate statistically significant differences between means at $P < 0.05$. For pH, the range of recorded values are presented.

Parameters	Treatments						
	C1	C2	C3	P1	P2	P3	C
pH	6.7 – 7.8	6.6 – 8.2	6.6 – 7.8	7.0 – 8.4	7.0 – 8.6	6.8 – 8.8	7.1 – 8.2
Dissolved oxygen (mg/ L)	6.81 ± 0.35^{ab}	7.14 ± 0.33^{ab}	5.23 ± 0.27^{c}	6.32 ± 0.21^{bc}	7.29 ± 0.26^{ab}	5.14 ± 0.24^{c}	7.71 ± 0.32^{a}
Free CO_2 (mg/ L)	4.58 ± 0.40^{a}	4.08 ± 0.25^{ab}	3.48 ± 0.19^{ab}	2.98 ± 0.29^{b}	2.96 ± 0.45^{b}	3.15 ± 0.41^{b}	3.09 ± 0.19^{b}
CO_3 alkalinity (mg/ L)	-	-	-	0.65 ± 0.65^{a}	1.32 ± 0.89^{a}	0.74 ± 0.74^{a}	-
HCO_3 alkalinity (mg/ L)	55.08 ± 2.38^{cd}	75.69 ± 4.53^{bcd}	93.54 ± 7.93^{ab}	64.73 ± 4.05^{cd}	78.19 ± 5.57^{bc}	106.23 ± 9.28^{a}	53.15 ± 1.74^{d}
PO_4 – P (mg/ L)	0.152 ± 0.016^{cd}	0.271 ± 0.024^{bc}	0.328 ± 0.038^{ab}	0.161 ± 0.014^{c}	0.301 ± 0.028^{ab}	0.410 ± 0.044^{a}	0.029 ± 0.002^{d}
NH_4 – N (mg/ L)	0.129 ± 0.014^{de}	0.181 ± 0.022^{cd}	0.400 ± 0.044^{ab}	0.287 ± 0.030^{bc}	0.321 ± 0.030^{ab}	0.429 ± 0.041^{a}	0.03 ± 0.002^{e}
NO_2 – N (mg/ L)	0.026 ± 0.003^{bc}	0.032 ± 0.003^{bc}	0.046 ± 0.004^{a}	0.023 ± 0.003^{cd}	0.039 ± 0.003^{ab}	0.053 ± 0.004^{a}	0.008 ± 0.001^{d}
NO_3 – N (mg/ L)	0.121 ± 0.011^{d}	0.30 ± 0.031^{bc}	0.412 ± 0.044^{ab}	0.162 ± 0.019^{cd}	0.329 ± 0.031^{b}	0.50 ± 0.061^{a}	0.03 ± 0.005^{d}
Specific Conductivity (mmhos/ cm)	0.431 ± 0.023^{cd}	0.572 ± 0.043^{bc}	0.688 ± 0.054^{b}	0.412 ± 0.025^{cd}	0.68 ± 0.056^{b}	1.151 ± 0.665^{a}	0.272 ± 0.007^{d}

Table 6 Species composition, abundance (no./ L) and relative abundance (% of total numbers) of plankton in culture tanks manured with cowdung and poultry excreta at different rates, and control. Each mean value represents data from 26 samples collected two times a week during the 3-month growth period (March – May, 2002).

Plankton	C1		C2		C3		P1		P2		P3		C	
	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)
Daphnia sp.	72.31	11.97	150.20	12.59	60.12	6.50	118.70	14.75	212.39	14.15	71.19	7.04	-	-
Moina sp.	81.20	13.44	191.66	16.07	123.20	13.31	120.12	14.93	254.12	16.93	126.12	12.48	-	-
Bosmina sp.	18.24	3.02	26.42	2.22	28.20	3.05	21.30	2.65	31.24	2.08	28.24	2.79	-	-
Cladocera	171.75	28.43	368.28	30.88	211.52	22.86	260.12	32.34	497.75	33.16	225.55	22.31	-	-
Cyclops sp.	180.12	29.81	310.21	26.01	251.50	27.18	214.29	26.64	386.52	25.75	292.55	28.94	152.14	49.04
Diaptomus sp.	118.36	19.59	260.24	21.82	182.12	19.68	164.12	20.41	290.24	19.34	198.21	19.61	80.06	25.80
Nauplii	50.24	8.32	94.26	7.90	62.24	6.73	58.13	7.23	112.12	7.47	62.45	6.18	43.12	13.90
Copepoda	348.72	57.72	664.71	55.73	495.86	53.58	436.54	54.28	788.88	52.56	553.21	54.72	275.32	88.74
Brachionus sp.	28.23	4.67	46.12	3.87	36.28	3.92	30.12	3.74	58.19	3.88	45.12	4.46	20.33	6.55
Keratella sp.	12.17	2.02	24.33	2.03	28.20	3.05	29.30	3.64	38.16	2.54	26.16	2.59	-	-
Rotifera	40.40	6.69	70.45	5.90	64.48	6.97	59.42	7.38	96.35	6.42	71.28	7.05	20.33	6.55
Chlorella sp.	6.24	1.03	8.71	0.73	24.96	2.70	6.11	0.76	16.88	1.12	26.60	2.63	-	-
Navicula sp.	16.79	2.78	30.82	2.58	42.14	4.55	17.30	2.15	42.51	2.83	54.38	5.38	4.24	1.37
Spirogyra sp.	9.02	1.49	11.35	0.95	27.90	3.01	7.15	0.89	12.65	0.84	28.20	2.79	4.22	1.36
Scenedesmus sp.	-	-	2.25	0.19	9.12	0.98	2.04	0.25	3.07	0.20	6.59	0.65	-	-
Phacus sp.	11.26	1.86	33.16	2.78	45.26	4.89	13.73	1.71	39.51	2.63	41.13	4.07	4.02	1.30
Synedra sp.	-	-	2.98	0.25	4.19	0.45	1.90	0.24	3.28	0.22	4.02	0.40	2.12	0.68
Phytoplankton	43.31	7.17	89.27	7.48	153.57	16.59	48.23	6.0	117.90	7.86	160.92	15.92	14.60	4.71
Total Plankton	604.18		1192.71		925.43		804.31		1500.88		1010.96		310.25	

Table 7 Growth parameters recorded for koi carp reared in concrete tanks manured with cowdung and poultry excreta, applied at different rates, and control for 3 months (March – May, 2002). Different superscripts in the same row indicate statistically significant differences ($P < 0.05$).

	Treatments						
	C1	**C2**	**C3**	**P1**	**P2**	**P3**	**C**
Manure applied		Cowdung			Poultry excreta		-
Application rate (kg/ m^3/ 10 days)	0.13	0.26	0.39	0.13	0.26	0.39	-
Harvest weight (g)	3.31 ± 0.05^{f}	7.47 ± 0.12^{b}	4.89 ± 0.13^{d}	4.28 ± 0.10^{e}	9.17 ± 0.07^{a}	6.47 ± 0.11^{c}	3.03 ± 0.04^{f}
Weight gain (g)	3.22 ± 0.05^{f}	7.38 ± 0.12^{b}	4.80 ± 0.13^{d}	4.19 ± 0.10^{e}	9.08 ± 0.07^{a}	6.38 ± 0.11^{c}	2.94 ± 0.04^{g}
SGR (%/ day)	4.01 ± 0.07^{f}	4.93 ± 0.08^{b}	4.44 ± 0.10^{d}	4.29 ± 0.06^{e}	5.14 ± 0.03^{a}	4.76 ± 0.04^{c}	3.94 ± 0.02^{g}
Survival rate (%)	70.5^{bc}	81.5^{a}	70.25^{bc}	71.75^{b}	86.0^{a}	70.25^{bc}	65.5^{c}

4.2. Studies on the effect of water exchange on fish production (Experiment No. 2)

4.2.1. Organic manure quality

The amount of total nitrogen and organic carbon in the poultry manure stood at 2.66% and 30.19%, respectively.

4.2.2. Water quality

Water temperature was between 24°C and 35°C during the 90 days. However, there was no difference in water temperature between the treatments on any given sampling day. The pH ranged from 6.2 to 8.6 (**Table 8**). Higher water exchange rates increased the average DO in WE1 (7.94 mg/ L), which was significantly higher ($P < 0.05$) than the other treatments. In contrast, the highest BOD values were obtained in NE (4.63 mg/ L; $P < 0.05$). The NE treatment also showed the highest concentrations of specific conductivity, NH_4 - N, NO_2 - N, NO_3 - N, PO_4 - P and HCO_3 alkalinity, that was significantly higher ($P < 0.05$) than the other treatments (**Table 8**). CO_3 alkalinity was observed only in WE1, WE2 and WE3 treatments, for very limited periods, when the free CO_2 content in these treatments was absent. Within the different treatments with water exchange (WE1, WE2, WE3 and WE4), there were not much significant differences ($P > 0.05$) between the average values of bicarbonate alkalinity, NH_4 - N, NO_2 - N and specific conductivity (**Table 8**).

4.2.3. Plankton abundance

On an average, plankton concentration was highest in WE1 treatment, followed in the decreasing order by WE2, WE3, WE4 and NE treatments ($P < 0.05$) (**Table 9**). The plankton volume primarily consisted of zooplankton. Phytoplankton accounted for 9.53% (WE1) to 46.08% (NE) of the total plankton content. The abundance (no./ L) of the different plankton groups also differed considerably. Average number of cladocerans in WE1 (597.09) was 462% the average number of the same group in the NE treatment (129.17). Copepoda was the most dominant zooplankton in all the treatments ranging from 47.65 % in WE1 to 53.91 % in NE (**Table 9**).

4.2.4. Fish growth and survival

At harvest, maximum weight gain of koi carp (**Table 10**) was achieved in the WE1 treatment, followed in the decreasing order by the WE2, WE3, WE4 and NE treatments ($P < 0.05$). The SGR was quite high (> 3.5) in all the treatments, though the differences among the various treatments were significant ($P < 0.05$). There was a significant difference ($P < 0.05$) in the survival of koi carp among the treatments, ranging from 60.43% (NE) to 95.21% (WE1).

4.2.5. Number of marketable fish

To determine the output of marketable fish, the percentage and number of fish above a total weight of 4 g was estimated from the probability distribution at the end of the study. The number of marketable fish was significantly higher in WE1 ($P < 0.05$), followed in decreasing order by WE2, WE3, WE4 and NE treatments (**Table 11**).

Table 8 Mean ± SE of the major water quality parameters analyzed for the five treatments. Each mean value represents 13 samples collected at weekly intervals during the 3-month growth period (June – August, 2002). Different superscripts in the same row indicate statistically significant differences between means at $P < 0.05$. For pH, the range of recorded values are presented.

	Treatments				
Parameters	**WE1**	**WE2**	**WE3**	**WE4**	**NE**
pH	6.8 – 8.6	6.8 – 8.6	6.8 – 8.5	6.5 – 8.4	6.2 – 8.2
Dissolved oxygen (mg/ L)	7.94 ± 0.32^{a}	7.37 ± 0.30^{ab}	6.91 ± 0.24^{bc}	6.08 ± 0.18^{c}	5.08 ± 0.26^{d}
Biological Oxygen Demand (mg/ L)	1.23 ± 0.17^{c}	1.46 ± 0.23^{bc}	1.99 ± 0.36^{bc}	2.62 ± 0.28^{b}	4.63 ± 0.52^{a}
Free CO_2 (mg/ L)	0.42 ± 0.15^{c}	0.49 ± 0.17^{c}	0.61 ± 0.20^{c}	1.86 ± 0.23^{b}	3.16 ± 0.46^{a}
CO_3 alkalinity (mg/ L)	0.66 ± 0.17^{a}	0.60 ± 0.19^{a}	0.13 ± 0.13^{a}	-	-
HCO_3 alkalinity (mg/ L)	95.19 ± 6.89^{b}	102.95 ± 8.42^{b}	110.07 ± 9.66^{ab}	115.5 ± 9.38^{ab}	146.25 ± 11.02^{a}
PO_4 – P (mg/ L)	0.226 ± 0.028^{b}	0.253 ± 0.031^{b}	0.294 ± 0.035^{b}	0.428 ± 0.061^{ab}	0.563 ± 0.080^{a}
NH_4 – N (mg/ L)	0.163 ± 0.021^{b}	0.183 ± 0.025^{b}	0.202 ± 0.027^{b}	0.308 ± 0.044^{b}	0.753 ± 0.148^{a}
NO_2 – N (mg/ L)	0.021 ± 0.005^{b}	0.026 ± 0.005^{b}	0.027 ± 0.006^{b}	0.038 ± 0.010^{b}	0.211 ± 0.048^{a}
NO_3 – N (mg/ L)	0.187 ± 0.022^{b}	0.217 ± 0.028^{b}	0.272 ± 0.033^{b}	0.390 ± 0.056^{ab}	0.608 ± 0.096^{a}
Specific Conductivity (mmhos/ cm)	0.586 ± 0.051^{b}	0.612 ± 0.053^{b}	0.672 ± 0.065^{b}	0.786 ± 0.072^{b}	1.156 ± 0.146^{a}

Table 9 Species composition, abundance (no./ L) and relative abundance (% of total numbers) of plankton in culture tanks manured with poultry excreta, under different water exchange regimes. Each mean value represents data from 25 samples collected two times a week during the 3 month growth period (June – August, 2002).

	WE1		WE2		WE3		WE4		NE	
Plankton	**(no. / L)**	**(%)**	**(no. / L)**	**(%)**	**(no. / L)**	**(%)**	**(no. / L)**	**(%)**	**(no. / L)**	**(%)**
Daphnia sp.	252.30	16.82	200.72	14.20	178.66	14.61	91.29	10.06	39.12	6.11
Moina sp.	276.24	18.41	258.22	18.27	194.52	15.91	128.24	14.14	66.05	10.32
Bosmina sp.	68.55	4.57	63.32	4.48	63.20	5.17	50.14	5.53	24.00	3.75
Cladocera	597.09	39.80	522.26	36.96	436.38	35.69	269.67	29.73	129.17	20.18
Cyclops sp.	324.05	21.60	368.24	26.06	272.14	22.26	184.62	20.36	95.12	14.86
Diaptomus sp.	252.44	16.83	205.12	14.52	196.02	16.03	126.55	13.95	60.28	9.42
Nauplii	70.12	4.67	66.24	4.69	66.16	5.41	44.28	4.88	22.14	3.46
Copepoda	646.61	43.10	639.60	45.27	534.32	43.70	355.45	39.19	177.54	27.73
Brachionus sp.	67.08	4.47	60.92	4.31	56.12	4.59	32.14	3.54	20.18	3.15
Keratella sp.	46.22	3.08	40.32	2.85	34.66	2.83	27.20	3.00	18.22	2.84
Rotifera	113.30	7.55	101.24	7.16	90.78	7.42	59.34	6.54	38.40	5.99
Chlorella sp.	30.12	2.01	29.12	2.06	26.24	2.14	40.16	4.43	52.12	8.14
Navicula sp.	38.24	2.55	42.12	2.98	40.76	3.33	43.71	4.82	56.11	8.76
Spirogyra sp.	25.12	1.67	23.06	1.63	31.12	2.54	51.16	5.64	68.24	10.66
Scenedesmus sp.	8.70	0.58	8.12	0.57	9.22	0.75	16.44	1.81	28.18	4.40
Phacus sp.	36.15	2.41	42.26	2.99	48.12	3.93	64.14	7.07	80.15	12.52
Synedra sp.	4.61	0.30	5.03	0.35	5.62	0.46	6.78	0.74	10.20	1.59
Phytoplankton	142.94	9.53	149.71	10.59	161.08	13.17	222.39	24.52	295.00	46.08
Total Plankton	1499.94	-	1412.81	-	1222.56	-	906.85	-	640.11	-

Table 10 Growth parameters recorded for koi carp reared in concrete tanks manured with poultry excreta under different water exchange regimes for 3 months (June – August, 2002). Different superscripts in the same row indicate statistically significantly differences ($P < 0.05$).

	Treatments				
	WE1	**WE2**	**WE3**	**WE4**	**NE**
Harvest weight (g)	9.56 ± 0.08^{a}	8.18 ± 0.08^{b}	7.39 ± 0.10^{b}	5.75 ± 0.08^{c}	3.01 ± 0.11^{d}
Weight gain (g)	9.47 ± 0.08^{a}	8.09 ± 0.08^{b}	7.30 ± 0.10^{b}	5.66 ± 0.08^{c}	2.91 ± 0.11^{d}
SGR (%/ day)	5.16 ± 0.05^{a}	5.08 ± 0.06^{b}	4.89 ± 0.12^{c}	4.61 ± 0.08^{d}	3.92 ± 0.03^{e}
Survival rate (%)	95.21^{a}	89.61^{b}	81.96^{c}	74.84^{d}	60.43^{e}

Table 11 Average number of marketable fish (those heavier than 4.0 g) produced, together with marketable fish produced, expressed as a percentage of total number of fish produced (A) and as a percentage of number of fish stocked (B) in the five treatments. Different superscripts in a column represent statistically significant differences ($P < 0.05$).

Treatments	Number of marketable fish produced (fish/ tank)	Marketable fish (%)	
		A	B
WE1	380 [a]	100	95.21
WE2	358 [b]	100	89.61
WE3	327 [c]	100	81.75
WE4	300 [d]	100	74.90
NE	0.05 [e]	0.020	0.012

4.3. Studies on the effect of stocking density on fish production (Experiment No. 3)

4.3.1. Water quality

Water temperature was between 17°C and 26°C during the 90 days. However, there was no difference in water temperature between the treatments on any given sampling day. The pH ranged from 5.8 to 7.4 (**Table 12**). There were marked differences in water quality among the treatments (**Table 12**). Values of DO were significantly lower in D4 and D5 ($P < 0.05$), compared to other treatments. CO_3 alkalinity was absent in all treatments during the entire study period. Average HCO_3 alkalinity, NH_4 - N, NO_2 - N, NO_3 - N and PO_4 - P were significantly higher ($P < 0.05$) in D4 and D5, compared to other treatments (**Table 12**). Specific conductivity was significantly higher in D5, than in other treatments ($P < 0.05$).

4.3.2. Fish growth and survival

Weight gain of koi carp was affected by the stocking density (**Table 13**). At harvest, maximum weight gain was achieved in D1, followed in decreasing order by D2, D3, D4 and D5 treatments ($P < 0.05$). The SGR was quite high (> 3.5) in all the treatments, though there were significant differences ($P < 0.05$) within the treatments (**Table 13**), except D2 and D3, where the SGR did not record significantly differently from each other ($P > 0.05$). The survival rates of fish ranged from 62.43% in D5 to 93.36% in D1. Calculated FCR values averaged 1.82 - 2.88 among the treatments, with the maximum value (2.88) recorded in D5, which had the highest stocking density (**Table 13**).

4.3.3. Number of marketable fish

To determine the output of marketable fish, the percentage and number of fish above a total weight of 4 g was estimated from the probability distribution at the end of the study. Although all the fish collected from the D1, D2, and D3 treatments could be marketed, since they achieved the minimum marketable size, the highest number of marketable fish was produced in D3 (**Table 14**), which had a stocking density of 0.3 fish/ L. When the fish were stocked at densities higher than 0.3 fish/ L, it was observed that the number as well as percentage of fish above the set marketable size (4 g) decreased (**Table 14**).

Table 12 Mean ± SE of major water quality parameters analyzed for the five treatments. Each mean value represents 14 samples collected at weekly internals during the 3-month growth period (September – November, 2002). Different superscripts in the same row indicate statistically significant differences between means at $P < 0.05$. For pH, the range of recorded values are presented.

	Treatments				
Parameters	**D1**	**D2**	**D3**	**D4**	**D5**
pH	6.9 – 7.4	6.7 – 7.4	6.6 – 7.3	6.1 – 7.1	5.8 – 7.1
Dissolved oxygen (mg/ L)	6.57 ± 0.15^{a}	6.15 ± 0.18^{ab}	5.42 ± 0.42^{bc}	5.24 ± 0.22^{c}	4.82 ± 0.26^{c}
Free CO_2 (mg/ L)	5.03 ± 0.18^{b}	5.17 ± 0.20^{b}	5.53 ± 0.23^{b}	6.67 ± 0.31^{a}	6.81 ± 0.31^{a}
HCO_3 alkalinity (mg/ L)	35.14 ± 0.77^{c}	37.65 ± 1.16^{c}	40.22 ± 1.45^{bc}	47.45 ± 2.33^{ab}	53.69 ± 3.02^{a}
NO_3 – N (mg/ L)	0.105 ± 0.006^{c}	0.151 ± 0.011^{bc}	0.172 ± 0.014^{b}	0.235 ± 0.018^{a}	0.263 ± 0.020^{a}
NO_2 – N (mg/ L)	0.019 ± 0.001^{b}	0.021 ± 0.001^{b}	0.023 ± 0.002^{b}	0.031 ± 0.003^{a}	0.034 ± 0.003^{a}
NH_4 – N (mg/ L)	0.145 ± 0.005^{c}	0.175 ± 0.008^{bc}	0.237 ± 0.016^{b}	0.357 ± 0.026^{a}	0.432 ± 0.037^{a}
PO_4 – P (mg/ L)	0.152 ± 0.011^{b}	0.182 ± 0.013^{b}	0.217 ± 0.016^{b}	0.312 ± 0.024^{a}	0.342 ± 0.024^{a}
Specific conductance (mmhos/ cm)	0.22 ± 0.002^{c}	0.23 ± 0.005^{c}	0.25 ± 0.008^{b}	0.31 ± 0.013^{b}	0.37 ± 0.018^{a}

Table 13 Growth parameters recorded for koi carp reared in concrete tanks at different stocking densities for 3 months (September – November, 2002). Different superscripts in the same row indicate statistically significant differences ($P < 0.05$).

	Treatments				
	D1	**D2**	**D3**	**D4**	**D5**
Harvest weight (g)	7.42 ± 0.05^{a}	6.69 ± 0.04^{b}	6.46 ± 0.03^{c}	4.12 ± 0.07^{d}	3.48 ± 0.05^{e}
Weight gain (g)	7.28 ± 0.05^{a}	6.55 ± 0.04^{b}	6.32 ± 0.03^{c}	3.98 ± 0.07^{d}	3.34 ± 0.05^{e}
SGR (%/ day)	4.38 ± 0.03^{a}	4.26 ± 0.02^{b}	4.22 ± 0.02^{b}	3.72 ± 0.04^{c}	3.54 ± 0.03^{d}
FCR	1.82 ± 0.02^{a}	2.03 ± 0.02^{b}	2.07 ± 0.03^{b}	2.83 ± 0.04^{c}	2.88 ± 0.03^{c}
Survival rate (%)	93.36^{a}	83.40^{b}	82.27^{b}	73.30^{c}	62.43^{d}

Table 14 Average number marketable fish (those heavier than 4.0 g) produced, together with marketable fish produced, expressed as a percentage of total number of fish produced (A) and total number of fish stocked (B) in the five treatments with different stocking densities. Different superscripts in a column represent statistically significant differences ($P < 0.05$).

Treatments	Stocking density		Number of marketable fish produced (fish/ tank)	Marketable fish (%)	
	(fish/ L)	(fish/ tank)		A	B
D1	0.1	15	14 [d]	100	93.36
D2	0.2	30	25 [c]	100	83.40
D3	0.3	45	37 [a]	100	82.27
D4	0.4	60	30.42 [b]	69.14	50.70
D5	0.5	75	0.05 [e]	0.11	0.07

4.4. Studies on the effect of live-food treatment on fish production against conventional manuring regimen. (A) Comparative account of fish production throughout different seasons (culture periods) in a year, namely, winter, summer, monsoon and post monsoon (Experiment No. 4)

4.4.1. Organic manure quality

The amount of total nitrogen in the cow manure used in the different seasonal trials ranged from 1.83% to 2.33%, while the poultry manure had 2.45% to 2.82% of total nitrogen. The amount of organic carbon in the cow and poultry manures ranged from 19.22% to 25.12% and 27.31% to 32.65%, respectively.

4.4.2. Water quality

Water temperature averaged 18.58°C, 29.67°C, 28.25°C and 26.5°C during the winter, summer, monsoon and post monsoon trials, respectively. Throughout the four seasonal trials, there was no difference in water temperature between the different treatments on any given sampling day. The variations of water temperature in the four seasonal trials are shown in **Figure 1**. The results of the various water quality parameters in the experimental tanks during the different seasonal trials are presented in **Table 15**. Values of DO were significantly higher in the LF and C treatments ($P < 0.05$) than the manured treatments in every season. Heavy rainfall during the monsoon trial increased the DO concentration in all the treatments (**Table 15**).

Values of free CO_2, BOD, total alkalinity, conductivity, NH_4 - N, NO_2 - N, NO_3 - N and PO_4 - P were significantly higher ($P < 0.05$) in the manured treatments (PM and CD), compared to the LF and C treatments in all seasonal trials. The concentrations of BOD, total alkalinity, NH_4 - N and NO_2 - N were reduced considerably during the monsoon trial in all the treatments (**Table 15**). However, the responses of NO_3 - N and PO_4 - P were opposite to NH_4 - N or NO_2 - N, and the highest values of NO_3 - N and PO_4 - P were recorded during the monsoon trial in all the treatments (**Table 15**).

4.4.3. Plankton abundance

Species composition, abundance (no./ L) and relative abundance (% of total numbers) of plankton in the different seasonal trials are presented in **Table(s) 16** (winter), **17** (summer), **18** (monsoon) and **19** (post monsoon). On an average, the plankton volume was highest in the PM treatment, followed in decreasing order by the LF, CD and C treatments in all seasonal trials ($P < 0.05$). However, the zooplankton concentration was highest in the LF treatment in all seasons (**Figure 2**). In all the treatments, abundance (no./ L) of zooplankton was lowest during winter. On the contrary, phytoplankton abundance (no./ L) was highest in all the treatments during winter (**Figure 2**).

4.4.4. Fish growth and survival

In all the seasonal trials, the average harvest weight of koi carp was highest ($P < 0.05$) in the LF treatment, followed in decreasing order by the PM, CD and C treatments (**Table 20**). In all the treatments, the average weight gain of carp during the winter trial was considerably lower than that achieved in the summer, monsoon or post monsoon trials (**Table 20**). The SGR was quite high (> 3.5) in all treatments throughout the four seasonal trials, though the differences among various treatments were significant ($P < 0.05$). Highest survival rates in all the seasonal trials were obtained in the LF treatment, followed in decreasing order by the PM, CD and C treatments ($P < 0.05$). The control treatment produced the highest number of deformed koi carp during all seasons, while the lowest numbers were recorded in the LF treatment ($P < 0.05$).

4.4.5. Number of marketable fish

The number and percentage of marketable koi carp in the four treatments, as estimated from the probability distribution at the end of each growth trial is presented in **Table 21**. In every season, the LF treatment produced the highest numbers of saleable fish ($P < 0.05$), while the control treatment yielded the lowest numbers. The number of marketable fish produced was lowest in the winter trial, when only one of the treatments (LF) produced fish of marketable size, while other treatments were unproductive (**Table 21**). Percentage of marketable fish produced in the manured treatments was considerably higher in the summer, monsoon and post monsoon trials, than in winter (**Table 21**).

Table 15 Average water quality parameters (SE in parentheses) in the four treatments recorded in the four growth experiments during different seasons throughout one year. Different superscripts of each water quality parameter in the same row indicate statistically significant differences between means at $P < 0.05$.

Culture period	Dissolved oxygen (mg/ L)				pH (mg/ L)			
	LF	PM	CD	C	LF	PM	CD	C
Winter	6.75^{a} (0.23)	5.76^{bc} (0.14)	5.26^{c} (0.15)	6.30^{ab} (0.18)	7.12^{a} (0.08)	6.26^{b} (0.11)	6.08^{b} (0.12)	6.99^{a} (0.06)
Summer	6.83^{a} (0.19)	5.82^{bc} (0.17)	5.37^{c} (0.20)	6.51^{ab} (0.23)	7.39^{a} (0.10)	6.82^{bc} (0.13)	6.44^{c} (0.16)	7.26^{ab} (0.10)
Monsoon	7.72^{a} (0.12)	6.94^{b} (0.13)	6.57^{b} (0.17)	7.47^{a} (0.10)	7.56^{a} (0.08)	6.64^{b} (0.14)	6.30^{b} (0.18)	7.28^{a} (0.08)
Post monsoon	6.87^{a} (0.18)	5.99^{bc} (0.17)	5.70^{c} (0.16)	6.46^{ab} (0.19)	7.12^{a} (0.07)	6.31^{b} (0.09)	5.95^{b} (0.12)	7.66^{a} (0.08)
	Free CO_2 (mg/ L)				BOD (mg/ L)			
	LF	PM	CD	C	LF	PM	CD	C
Winter	2.34^{c} (0.10)	3.12^{ab} (0.18)	3.64^{a} (0.24)	2.57^{bc} (0.16)	1.11^{b} (0.08)	3.11^{a} (0.31)	2.56^{a} (0.28)	1.23^{b} (0.08)
Summer	1.37^{c} (0.13)	2.23^{b} (0.17)	2.97^{a} (0.27)	1.63^{bc} (0.12)	0.94^{b} (0.01)	2.40^{a} (0.29)	1.99^{a} (0.21)	1.14^{b} (0.10)
Monsoon	1.21^{b} (0.08)	1.77^{ab} (0.17)	2.34^{a} (0.25)	1.41^{b} (0.12)	0.63^{b} (0.06)	1.59^{a} (0.16)	1.22^{a} (0.11)	0.70^{b} (0.07)
Post monsoon	0.96^{c} (0.08)	1.70^{ab} (0.11)	2.03^{a} (0.16)	1.31^{bc} (0.07)	1.36^{c} (0.12)	3.38^{a} (0.34)	2.50^{b} (0.25)	1.51^{c} (0.12)
	$NH_4 - N$ (mg/ L)				$NO_2 - N$ (mg/ L)			
	LF	PM	CD	C	LF	PM	CD	C
Winter	0.082^{b} (0.012)	0.351^{a} (0.041)	0.239^{a} (0.038)	0.10^{b} (0.014)	0.004^{b} (0.001)	0.026^{a} (0.003)	0.016^{ab} (0.002)	0.006^{b} (0.001)
Summer	0.084^{b} (0.009)	0.384^{a} (0.04)	0.321^{a} (0.037)	0.102^{b} (0.098)	0.008^{b} (0.001)	0.037^{a} (0.004)	0.026^{a} (0.003)	0.013^{b} (0.001)
Monsoon	0.06^{b} (0.003)	0.308^{a} (0.044)	0.208^{a} (0.025)	0.069^{b} (0.007)	0.003^{b} (0.001)	0.01^{a} (0.001)	0.007^{a} (0.001)	0.004^{b} (0.001)
Post monsoon	0.091^{c} (0.008)	0.452^{a} (0.051)	0.294^{b} (0.032)	0.144^{c} (0.013)	0.009^{b} (0.001)	0.044^{a} (0.004)	0.029^{a} (0.003)	0.012^{b} (0.001)
	$NO_3 - N$ (mg/ L)				$PO_4 - P$ (mg/ L)			
	LF	PM	CD	C	LF	PM	CD	C
Winter	0.042^{b} (0.006)	0.178^{a} (0.023)	0.14^{a} (0.017)	0.06^{b} (0.007)	0.08^{c} (0.007)	0.37^{a} (0.039)	0.24^{b} (0.030)	0.11^{c} (0.014)
Summer	0.061^{b} (0.006)	0.226^{a} (0.034)	0.166^{a} (0.025)	0.075^{b} (0.008)	0.16^{b} (0.020)	0.50^{a} (0.065)	0.40^{a} (0.046)	0.18^{b} (0.018)
Monsoon	0.072^{b} (0.008)	0.329^{a} (0.045)	0.235^{a} (0.028)	0.086^{b} (0.009)	0.20^{c} (0.012)	0.59^{a} (0.06)	0.43^{b} (0.046)	0.25^{c} (0.02)
Post monsoon	0.08^{c} (0.009)	0.193^{a} (0.024)	0.153^{ab} (0.015)	0.097^{bc} (0.009)	0.16^{b} (0.014)	0.55^{a} (0.063)	0.41^{a} (0.043)	0.19^{b} (0.015)
	Alkalinity (mg/ L)				Specific conductivity (mmhos/ cm)			
	LF	PM	CD	C	LF	PM	CD	C
Winter	17.75^{b} (1.14)	48.16^{a} (4.89)	36.33^{a} (3.91)	23.16^{b} (2.34)	0.231^{b} (0.011)	0.462^{a} (0.03)	0.458^{a} (0.04)	0.254^{b} (0.011)
Summer	26.16^{c} (2.26)	60.83^{a} (6.40)	48.66^{ab} (4.74)	33.66^{bc} (3.28)	0.178^{b} (0.017)	0.414^{a} (0.049)	0.404^{a} (0.046)	0.221^{b} (0.017)
Monsoon	11.66^{b} (0.77)	31.75^{a} (2.75)	27.16^{a} (2.47)	14.75^{b} (1.11)	0.171^{c} (0.01)	0.384^{a} (0.04)	0.302^{ab} (0.031)	0.19^{bc} (0.022)
Post monsoon	22.33^{b} (1.38)	60.50^{a} (5.77)	52.33^{a} (4.64)	26.50^{b} (2.28)	0.237^{b} (0.023)	0.59^{a} (0.044)	0.532^{a} (0.031)	0.281^{b} (0.025)

Table 16 Species composition, abundance (no./ L) and relative abundance (% of total numbers) of plankton in culture tanks maintained under different management regimes in the winter trial (4 December, 2002 – 19 February, 2003). Each mean value represents data from 22 samples collected two times a week during the 11-week growth period.

Plankton	LF		PM		CD		C	
	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)
Daphnia sp.	180.21	20.24	60.14	6.05	51.22	6.32	-	-
Moina sp.	208.97	23.48	121.04	12.39	88.32	10.90	-	-
Ceriodaphnia sp.	128.16	14.48	71.12	7.09	66.20	8.18	-	-
Bosmina sp.	21.07	2.36	25.22	2.83	20.14	2.48	-	-
Cladocera	538.41	60.56	277.52	28.36	225.88	27.88	-	-
Cyclops sp.	151.04	16.97	196.22	19.16	152.12	18.79	57.53	35.12
Diaptomus sp.	-	-	56.20	5.75	44.74	5.54	21.08	12.87
Nauplii	134.71	15.13	161.52	16.53	140.30	17.39	66.17	40.31
Copepoda	285.75	32.10	413.94	41.44	337.16	41.72	144.78	88.30
Brachionus sp.	6.24	0.70	41.24	4.22	36.10	4.46	0.33	0.20
Keratella sp.	5.40	0.58	40.22	4.11	28.87	3.56	-	-
Rotifera	11.64	1.28	81.46	8.33	64.97	8.02	0.33	0.20
Chlorella sp.	6.31	0.70	31.04	3.17	28.26	3.49	2.12	1.29
Navicula sp.	26.24	2.94	54.12	5.54	46.39	5.73	16.56	10.20
Spirogyra sp.	5.21	0.58	42.72	4.37	38.14	4.71	-	-
Scenedesmus sp.	-	-	6.20	0.63	5.89	0.72	-	-
Phacus sp.	12.16	1.36	61.68	6.31	56.30	6.95	-	-
Synedra sp.	4.21	0.47	8.19	0.83	6.20	0.76	-	-
Phytoplankton	54.13	6.05	203.95	20.85	181.18	22.36	18.68	11.49
Total Plankton	889.93	-	976.87	-	809.19	-	163.79	-

Table 17 Species composition, abundance (no./ L) and relative abundance (% of total numbers) of plankton in culture tanks maintained under different management regimes in the summer trial (4 March, 2003 – 20 May, 2003). Each mean value represents data from 22 samples collected two times a week during the 11-week growth period.

Plankton	LF		PM		CD		C	
	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)
Daphnia sp.	201.16	18.81	78.11	6.85	62.14	6.68	-	-
Moina sp.	239.72	22.41	142.32	12.48	116.21	12.40	-	-
Ceriodaphnia sp.	180.24	16.85	82.14	7.20	74.18	7.91	-	-
Bosmina sp.	26.14	2.48	23.42	2.05	18.29	1.95	-	-
Cladocera	647.26	60.55	325.99	28.58	270.82	28.94	-	-
Cyclops sp.	182.12	17.03	261.09	22.98	217.76	23.24	81.31	32.55
Diaptomus sp.	12.05	1.12	91.73	8.04	63.24	6.75	34.10	13.65
Nauplii	161.07	15.06	196.55	17.24	170.95	18.24	101.62	40.68
Copepoda	355.24	33.21	549.37	48.26	451.95	48.23	217.03	86.88
Brachionus sp.	18.23	1.70	56.20	4.93	48.18	5.14	7.14	2.85
Keratella sp.	15.40	1.44	52.46	4.60	36.73	3.92	8.30	3.32
Rotifera	33.63	3.14	108.66	9.53	84.91	9.06	15.44	6.17
Chlorella sp.	4.26	0.39	20.12	1.76	19.76	2.10	3.12	1.26
Navicula sp.	16.32	1.52	42.20	3.70	30.95	3.30	14.20	5.68
Spirogyra sp.	-	-	30.91	2.71	26.08	2.78	-	-
Scenedesmus sp.	-	-	7.15	0.62	5.20	0.55	-	-
Phacus sp.	12.68	1.18	48.09	4.21	41.64	4.44	-	-
Synedra sp.	-	-	7.16	0.62	5.57	0.59	-	-
Phytoplankton	33.26	3.09	155.63	13.62	129.20	13.76	17.32	6.94
Total Plankton	1069.39	-	1139.65	-	936.88	-	249.79	-

Table 18 Species composition, abundance (no./ L) and relative abundance (% of total numbers) of plankton in culture tanks maintained under different management regimes in the monsoon trial (3 June, 2003 – 19 August, 2003). Each mean value represents data from 22 samples collected two times a week during the 11-week growth period.

Plankton	LF		PM		CD		C	
	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)
Daphnia sp.	185.10	18.80	62.16	5.83	54.25	6.00	-	-
Moina sp.	216.72	22.09	129.14	11.92	104.60	11.56	-	-
Ceriodaphnia sp.	154.24	15.72	84.16	7.78	76.08	8.44	-	-
Bosmina sp.	22.05	2.25	24.17	2.23	18.12	2.00	-	-
Cladocera	578.11	58.86	299.62	27.76	253.05	28.00	-	-
Cyclops sp.	162.04	16.50	240.22	22.17	221.30	24.50	69.24	32.08
Diaptomus sp.	16.32	1.66	88.75	8.19	64.12	7.09	28.70	13.29
Nauplii	154.26	15.82	191.09	17.63	160.24	17.72	96.99	44.94
Copepoda	332.62	33.98	520.06	47.99	445.66	49.31	194.93	90.31
Brachionus sp.	16.24	1.65	58.02	5.35	49.63	5.48	2.14	0.99
Keratella sp.	14.22	1.44	56.91	5.25	38.14	4.21	3.21	1.50
Rotifera	30.46	3.09	114.93	10.60	87.77	9.69	5.35	2.49
Chlorella sp.	5.12	0.52	18.14	1.60	16.25	1.79	1.25	0.57
Navicula sp.	20.34	2.07	43.12	3.98	34.08	3.76	14.29	6.62
Spirogyra sp.	-	-	32.14	2.96	24.08	2.66	-	-
Scenedesmus sp.	-	-	7.04	0.64	3.12	0.34	-	-
Phacus sp.	14.35	1.46	42.05	3.88	34.19	3.78	-	-
Synedra sp.	-	-	6.24	0.57	5.91	0.65	-	-
Phytoplankton	39.81	4.05	148.73	13.63	117.63	12.98	15.54	7.19
Total Plankton	981.0	-	1083.34	-	904.11	-	215.82	-

Table 19 Species composition, abundance (no./ L) and relative abundance (% of total numbers) of plankton in culture tanks maintained under different management regimes in the post monsoon trial (2 September, 2003 – 18 November, 2003). Each mean value represents data from 22 samples collected two times a week during the 11-week growth period.

Plankton	LF		PM		CD		C	
	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)
Daphnia sp.	198.12	18.60	79.12	6.93	64.26	6.90	-	-
Moina sp.	230.24	21.61	136.72	11.98	120.09	12.92	-	-
Ceriodaphnia sp.	172.95	16.23	80.19	7.02	76.21	8.19	-	-
Bosmina sp.	25.71	2.45	28.24	2.47	22.50	2.44	-	-
Cladocera	627.02	58.89	324.27	28.40	283.06	30.45	-	-
Cyclops sp.	185.11	17.38	256.09	22.44	212.05	22.78	85.16	35.54
Diaptomus sp.	11.29	1.06	96.24	8.42	56.12	6.03	24.26	10.12
Nauplii	170.20	15.98	206.12	18.08	168.67	18.12	106.04	44.23
Copepoda	366.60	34.42	558.45	48.94	436.84	46.93	215.46	89.89
Brachionus sp.	18.90	1.77	54.32	4.76	47.22	5.07	5.16	2.15
Keratella sp.	17.23	1.61	48.54	4.25	39.14	4.20	2.54	1.06
Rotifera	36.13	3.38	102.86	9.01	86.36	9.27	7.70	3.21
Chlorella sp.	4.88	0.45	24.26	2.12	21.80	2.34	2.62	1.09
Navicula sp.	18.09	1.69	40.62	3.60	29.14	3.13	13.92	5.80
Spirogyra sp.	-	-	30.20	2.64	22.14	2.37	-	-
Scenedesmus sp.	-	-	8.16	0.71	4.68	0.50	-	-
Phacus sp.	12.26	1.15	45.14	3.95	40.20	4.32	-	-
Synedra sp.	-	-	7.12	0.62	6.29	0.67	-	-
Phytoplankton	35.23	3.29	155.50	13.64	124.25	13.33	16.54	6.89
Total Plankton	1064.98	-	1141.08	-	930.51	-	239.70	-

Table 20 Summary of fish growth parameters of koi carp produced in different treatments during the four seasonal trials throughout the year. Different superscripts in the same row indicate statistically significant differences ($P < 0.05$).

	Treatments			
	LF	**PM**	**CD**	**C**
Initial weight (g ± SE)				
Winter	0.12 ± 0.015 [a]	0.12 ± 0.015 [a]	0.12 ± 0.015 [a]	0.12 ± 0.015 [a]
Summer	0.13 ± 0.010 [a]	0.13 ± 0.010 [a]	0.13 ± 0.010 [a]	0.13 ± 0.010 [a]
Monsoon	0.14 ± 0.012 [a]	0.14 ± 0.012 [a]	0.14 ± 0.012 [a]	0.14 ± 0.012 [a]
Post monsoon	0.13 ± 0.015 [a]	0.13 ± 0.015 [a]	0.13 ± 0.015 [a]	0.13 ± 0.015 [a]
Harvest weight (g ± SE)				
Winter	4.56 ± 0.10 [a]	3.52 ± 0.07 [b]	2.86 ± 0.13 [c]	2.13 ± 0.08 [d]
Summer	8.32 ± 0.13 [a]	5.14 ± 0.18 [b]	4.22 ± 0.08 [c]	3.32 ± 0.06 [d]
Monsoon	6.01 ± 0.16 [a]	4.42 ± 0.08 [b]	4.04 ± 0.07 [c]	2.94 ± 0.05 [d]
Post monsoon	7.12 ± 0.26 [a]	4.88 ± 0.10 [b]	4.28 ± 0.25 [c]	3.61 ± 0.27 [d]
Weight gain (g ± SE)				
Winter	4.44 ± 0.10 [a]	3.40 ± 0.07 [b]	2.74 ± 0.13 [c]	2.01 ± 0.08 [d]
Summer	8.19 ± 0.13 [a]	5.01 ± 0.18 [b]	4.09 ± 0.08 [c]	3.19 ± 0.06 [d]
Monsoon	5.87 ± 0.16 [a]	4.28 ± 0.08 [b]	3.90 ± 0.07 [c]	2.80 ± 0.05 [d]
Post monsoon	6.99 ± 0.26 [a]	4.75 ± 0.10 [b]	4.15 ± 0.25 [c]	3.48 ± 0.27 [d]
SGR (%/ day)				
Winter	4.73 ± 0.12 [a]	4.39 ± 0.07 [b]	4.12 ± 0.03 [c]	3.73 ± 0.05 [d]
Summer	5.40 ± 0.08 [a]	4.78 ± 0.03 [b]	4.52 ± 0.05 [c]	4.21 ± 0.03 [d]
Monsoon	4.87 ± 0.04 [a]	4.48 ± 0.10 [b]	4.36 ± 0.08 [c]	3.94 ± 0.06 [d]
Post monsoon	5.19 ± 0.11 [a]	4.70 ± 0.05 [b]	4.53 ± 0.05 [c]	4.31 ± 0.09 [d]
Deformed individuals (%)				
Winter	4.16 [d]	6.17 [c]	7.23 [b]	15.17 [a]
Summer	1.16 [d]	4.67 [c]	5.33 [b]	14.33 [a]
Monsoon	2.83 [d]	5.0 [c]	7.67 [b]	14.84 [a]
Post monsoon	1.16 [d]	5.33 [c]	5.34 [b]	14.50 [a]
Survival (%)				
Winter	95.50 [a]	83.50 [b]	77.0 [c]	70.50 [d]
Summer	97.50 [a]	87.66 [b]	84.0 [c]	73.16 [d]
Monsoon	96.83 [a]	90.66 [b]	85.33 [c]	78.83 [d]
Post monsoon	97.66 [a]	91.16 [b]	85.17 [c]	77.98 [d]

Table 21 Average number of marketable fish (those heavier than 4.0 g) produced* during the different seasonal trials throughout the year, together with marketable fish produced expressed as a percentage of total number of fish produced* (A) and as a percentage of number of fish stocked (B) in the four treatments. Different superscripts in the same row indicate statistically significant differences between means ($P < 0.05$).

	Treatments			
	LF	**PM**	**CD**	**C**
Winter				
Number of marketable fish produced* (fish/ tank)	548 [a]	0 [b]	0 [b]	0 [b]
Marketable fish (%)				
A*	95.64 [a]	0 [b]	0 [b]	0 [b]
B	91.33 [a]	0 [b]	0 [b]	0 [b]
Summer				
Number of marketable fish produced* (fish/ tank)	578 [a]	498 [b]	413.73 [c]	0 [d]
Marketable fish (%)				
A*	98.80 [a]	94.67 [b]	82.09 [c]	0 [d]
B	96.33 [a]	83.0 [b]	68.96 [c]	0 [d]
Monsoon				
Number of marketable fish produced* (fish/ tank)	564 [a]	508.95 [b]	279 [c]	0 [d]
Marketable fish (%)				
A*	97.07 [a]	93.56 [b]	54.49 [c]	0 [d]
B	94.0 [a]	84.83 [b]	46.50 [c]	0 [d]
Post monsoon				
Number of marketable fish produced* (fish/ tank)	579 [a]	514 [b]	416.08 [c]	28.31 [d]
Marketable fish (%)				
A*	98.80 [a]	93.96 [b]	81.42 [c]	6.05 [d]
B	96.50 [a]	85.66 [b]	69.35 [c]	4.72 [d]

* Excluding deformed fish.

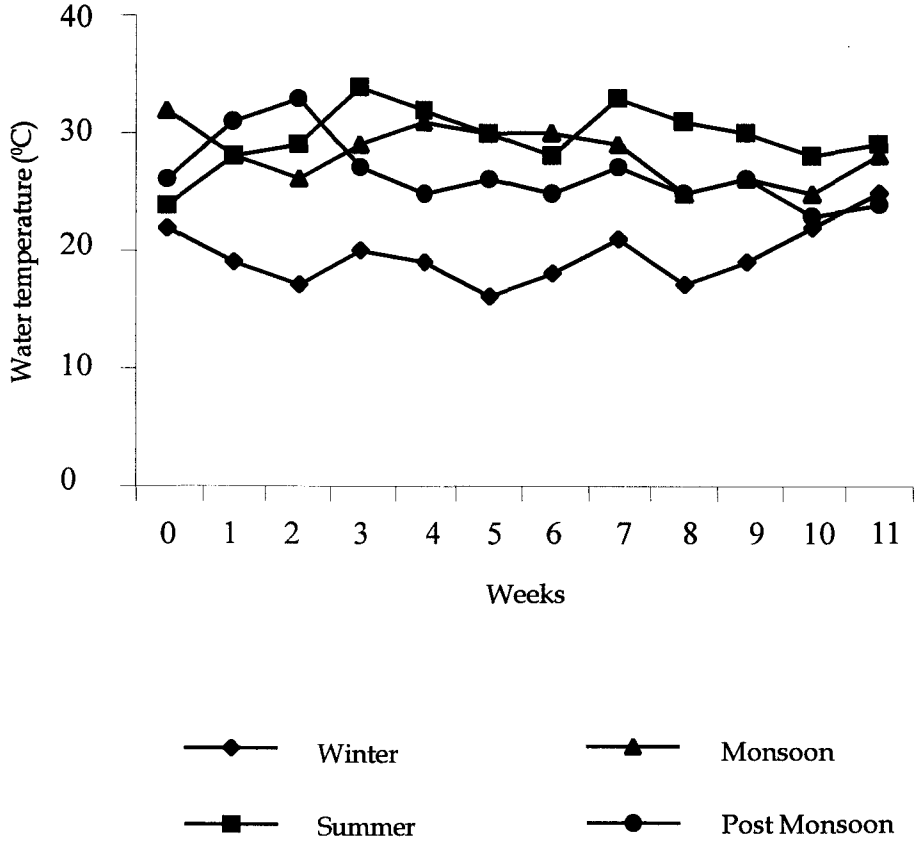

Figure 1 Weekly mean water temperature (°C) recorded from fish culture tanks at 0700 - 0800 hrs during the different seasonal trials.

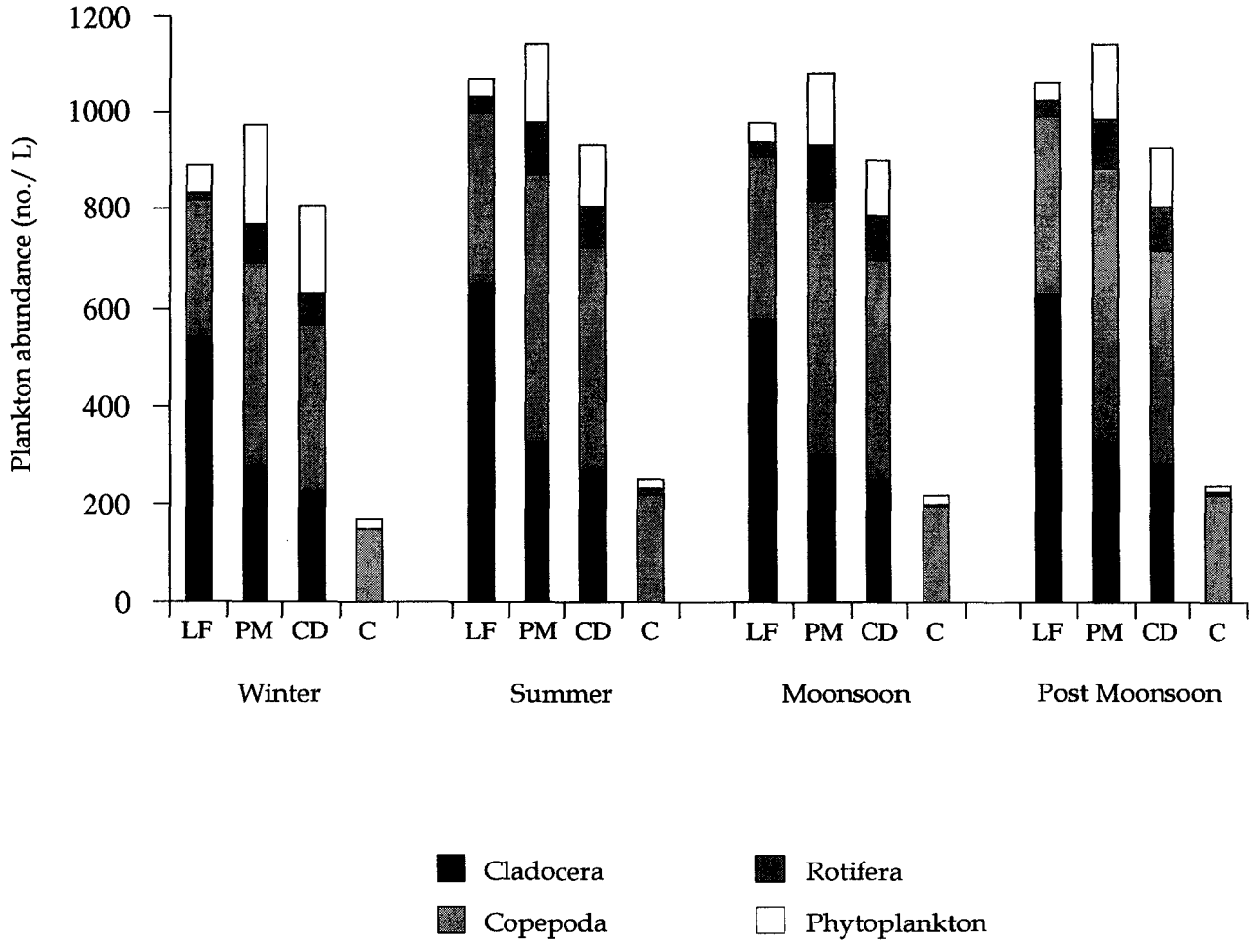

Figure 2 Plankton abundance (no./ L) in the four treatments during the different seasonal trials.

4.5. Studies on the effect of live-food treatment on fish production against conventional manuring regimen. (B) Comparative account of fish production in different culture systems, namely, concrete tanks and earthen ponds (Experiment No. 5)

4.5.1. Organic manure quality

The amount of total nitrogen in the cow and poultry manures was 2.20% and 2.52%, respectively, whereas, the amount of organic carbon was 23.17% and 29.66%, respectively.

4.5.2. Water quality

Water temperature was between 15°C and 24°C during the study period. However, there was no difference in water temperature between the different treatments on any given sampling day. The results of various water quality parameters are presented in **Table 22**. Significantly higher ($P < 0.05$) values of DO were obtained in the live-food and control treatments (for both tanks and ponds), compared to the manured treatments (**Table 22**). The total alkalinity here refers to HCO_3 alkalinity, as CO_3 was absent in any of the experimental units during the entire study period. The concentration of total alkalinity, BOD, PO_4 - P, NO_3 - N and specific conductivity were significantly higher ($P < 0.05$) in PPM and PCD, compared to other treatments. However, NO_2 - N and NH_4 - N were considerably higher ($P < 0.05$) in the manured treatments maintained in tanks (TPM and TCD), than other treatments (**Table 22**). Overall, the live-food treatments (TLF and PLF) yielded the lowest values ($P < 0.05$) of BOD and nutrients throughout the study period (**Table 22**).

4.5.3. Plankton abundance

Examination of plankton showed considerable differences in species diversity and abundance between different treatments. On an average, plankton abundance (no./ L) was highest in PPM, followed in decreasing order by PLF, PCD, TPM, TLF, TCD, PC and TC treatments ($P < 0.05$; **Table 23**). Zooplankton abundance (no./ L) was significantly greater ($P < 0.05$) in the live-food treatments, than in the poultry manured

treatments (**Table 23**), although, the later treatments (TPM and PPM) yielded significantly higher concentration ($P < 0.05$) of total plankton (zooplankton plus phytoplankton), compared to the live-food treatments (TLF and PLF, respectively). Cladocerans, which formed a substantial proportion of the total plankton composition in the live-food and manured units, were either absent or present in very low numbers in the control units (TC and PC, respectively). The copepoda was the most dominant group in all treatments (**Table 23**), except the live-food treatments (TLF and PLF), where cladocerans were more abundant. The phytoplankton concentrations in the manured treatments were significantly higher ($P < 0.05$) than the live-food and control treatments (**Table 23**).

4.5.4. Fish growth and survival

At harvest, maximum weight gain of koi carp (**Table 24**) was achieved in the PLF treatment, followed in decreasing order by TLF, PPM, PCD, TPM, TCD, PC and TC treatments ($P < 0.05$). The SGR was quite high (> 3.0) in all treatments, though the differences among various treatments were significant ($P < 0.05$). There was a significant difference ($P < 0.05$) in survival of koi carp among the treatments, ranging from 67.83% in TC to 95.50% in PLF (**Table 24**). The percentage of deformed koi carp was significantly higher ($P < 0.05$) in TC and PC, compared to other treatments (**Table 24**).

4.5.5. Number of marketable fish

The output of marketable fish was highest in the PLF treatment (**Table 25**), followed in decreasing order by TLF, PPM and PCD treatments ($P < 0.05$). The TPM, TCD, TC and PC treatments appeared to be unproductive, since none of the fish produced in these treatments attained marketable size (**Table 25**).

Table 22 Mean ± SE of major water quality parameters analyzed for the eight treatments. Each mean value represents 12 samples collected at weekly intervals during the 11-week growth period (2 December, 2003 – 17 February, 2004). Different superscripts in the same row indicate statistically significant differences between means at $P < 0.05$. For pH, the range of recorded values are presented.

Parameters	Treatments							
	TLF	PLF	TPM	PPM	TCD	PCD	TC	PC
pH	6.5 – 7.9	5.9 – 7.8	5.6 – 7.0	4.6 – 6.4	5.0 – 6.8	4.2 – 6.3	6.4 – 7.9	5.4 – 7.7
Dissolved oxygen (mg/ L)	7.23 ± 0.12 ^{a}	6.47 ± 0.22 ^{abc}	5.17 ± 0.22 ^{e}	5.01 ± 0.21 ^{e}	5.66 ± 0.20 ^{cde}	5.32 ± 0.22 ^{de}	6.96 ± 0.14 ^{ab}	6.11 ± 0.26 s^{bcd}
BOD (mg/ L)	1.22 ± 0.05 ^{d}	2.02 ± 0.09 ^{b}	2.32 ± 0.19 ^{b}	4.04 ± 0.25 ^{a}	2.0 ± 0.15 ^{bc}	3.46 ± 0.23 ^{a}	1.44 ± 0.08 ^{cd}	2.37 ± 0.13 ^{b}
Free CO_2 (mg/ L)	2.18 ± 0.08 ^{cd}	2.06 ± 0.01^{d}	3.10 ± 0.14 ^{a}	3.11 ± 0.17 ^{a}	2.87 ± 0.13 ^{ab}	2.72 ± 0.14 ^{abc}	2.42 ± 0.10 ^{bcd}	2.26 ± 0.08 ^{cd}
Total alkalinity (mg/ L)	37.17 ± 2.20 ^{c}	39.33 ± 1.80 ^{c}	62.17 ± 4.06 ^{b}	77.83 ± 4.27 ^{a}	57.50 ± 3.60 ^{b}	69.82 ± 3.48 ^{ab}	43.50 ± 2.70 ^{c}	43.42 ± 1.51^{c}
PO_4 – P (mg/ L)	0.121 ± 0.013 ^{e}	0.160 ± 0.009 ^{e}	0.463 ± 0.051 ^{bc}	0.731 ± 0.059 ^{a}	0.350 ± 0.040 ^{cd}	0.602 ± 0.053 ^{ab}	0.148 ± 0.012 ^{e}	0.180 ± 0.018 ^{de}
NH_4 – N (mg/ L)	0.142 ± 0.010 ^{de}	0.071 ± 0.008 ^{e}	0.641 ± 0.054 ^{a}	0.250 ± 0.011 ^{cd}	0.480 ± 0.038 ^{b}	0.202 ± 0.021 ^{de}	0.362 ± 0.033 ^{bc}	0.234 ± 0.019 ^{cd}
NO_2 – N (mg/ L)	0.012 ± 0.003 ^{c}	0.007 ± 0.001^{c}	0.041 ± 0.056 ^{a}	0.022 ± 0.002 ^{bc}	0.033 ± 0.026 ^{ab}	0.016 ± 0.001 ^{bc}	0.014 ± 0.005 ^{c}	0.009 ± 0.001 ^{c}
NO_3 – N (mg/ L)	0.068 ± 0.009 ^{d}	0.141 ± 0.008 ^{bcd}	0.218 ± 0.022 ^{b}	0.420 ± 0.032 ^{a}	0.167 ± 0.014 ^{bc}	0.354 ± 0.024 ^{a}	0.095 ± 0.012 ^{cd}	0.161 ± 0.011 ^{bcd}
Specific conductivity (mmhos/ cm)	0.20 ± 0.005 ^{d}	0.36 ± 0.014 ^{c}	0.43 ± 0.021 ^{c}	0.68 ± 0.041 ^{a}	0.37 ± 0.020 ^{c}	0.55 ± 0.034 ^{b}	0.22 ± 0.005 ^{d}	0.37 ± 0.013 ^{c}

Table 23 Species composition and abundance (no./ L) of plankton in culture tanks and ponds maintained under different management regimes. Each mean value represents data from 22 samples collected two times a week during the 11-week growth period (2 December, 2003 – 17 February, 2004).

Plankton	TLF (no./ L)	PLF (no./ L)	TPM (no./ L)	PPM (no./ L)	TCD (no./ L)	PCD (no./ L)	TC (no./ L)	PC (no./ L)
Daphnia sp.	195.20	225.12	66.14	80.09	44.14	56.42	-	4.05
Moina sp.	220.08	254.10	112.75	149.39	96.32	120.12	-	10.73
Ceriodaphnia sp.	143.02	161.34	74.03	97.36	63.47	82.07	-	5.32
Bosmina sp.	24.17	28.24	20.72	34.61	21.06	25.16	-	2.60
Cladocera	582.47	668.80	273.64	361.45	223.99	283.77	-	22.70
Cyclops sp.	146.28	190.13	206.33	280.11	170.21	232.49	72.09	116.23
Diaptomus sp.	-	20.14	86.12	116.09	60.16	74.36	25.14	40.12
Nauplii	122.14	151.25	158.04	182.12	136.52	168.63	70.15	92.70
Copepoda	268.42	361.52	450.49	578.32	366.89	475.42	167.38	249.05
Brachionus sp.	7.21	20.26	40.39	46.22	29.36	38.16	7.58	16.13
Keratella sp.	6.52	16.22	31.18	34.52	17.49	28.12	4.56	17.37
Rotifera	13.73	36.48	71.57	80.74	46.85	66.28	12.14	33.50
Chlorella sp.	4.24	16.12	19.12	40.39	17.12	37.12	2.56	17.05
Navicula sp.	21.02	28.25	42.25	81.06	35.24	66.24	18.37	26.24
Spirogyra sp.	-	12.14	34.12	46.12	32.26	45.23	-	14.55
Scenedesmus sp.	-	0.24	5.78	8.12	5.56	7.24	-	2.76
Phacus sp.	8.19	15.29	56.12	74.20	36.34	50.81	-	16.12
Synedra sp.	-	3.16	5.12	6.36	3.12	6.52	-	5.02
Phytoplankton	33.45	75.20	162.51	256.25	129.64	213.16	20.93	81.74
Total Plankton	898.07	1142.0	958.21	1276.76	803.37	1038.63	201.45	386.99

Table 24 Growth parameters recorded for koi carp reared in earthen ponds and concrete tanks under different management regimes for 11 weeks (2 December, 2003 – 17 February, 2004). Different superscripts in the same row indicate statistically significant differences ($P < 0.05$).

	Treatments							
	TLF	**PLF**	**TPM**	**PPM**	**TCD**	**PCD**	**TC**	**PC**
Harvest weight (g)	4.51 ± 0.10^{b}	6.02 ± 0.14^{a}	2.82 ± 0.04^{e}	4.09 ± 0.08^{c}	2.75 ± 0.04^{e}	3.72 ± 0.18^{d}	1.62 ± 0.03^{g}	2.02 ± 0.05^{f}
Weight gain (g)	4.39 ± 0.10^{b}	5.90 ± 0.14^{a}	2.70 ± 0.04^{e}	3.97 ± 0.08^{c}	2.63 ± 0.04^{f}	3.60 ± 0.18^{d}	1.50 ± 0.14^{h}	1.90 ± 0.05^{g}
SGR (%/ day)	4.71 ± 0.10^{b}	5.09 ± 0.14^{a}	4.10 ± 0.07^{e}	4.58 ± 0.09^{c}	4.09 ± 0.06^{e}	4.45 ± 0.08^{d}	3.38 ± 0.08^{g}	3.66 ± 0.05^{f}
Survival (%)	92.17^{b}	95.50^{a}	81.16^{d}	86.73^{c}	74.33^{e}	81.50^{d}	67.83^{f}	75.17^{e}
Deformed individuals (%)	4.83^{e}	2.43^{f}	6.80^{d}	4.50^{e}	7.66^{c}	5.16^{e}	21.33^{a}	12.83^{b}

Table 25 Average number of marketable fish (those heavier than 4.0 g) produced* in the experimental tanks and ponds, together with marketable fish produced, expressed as a percentage of total number of fish produced* (A) and as a percentage of number of fish stocked (B) in the different treatments. Different superscripts in a column represent statistically significant differences ($P < 0.05$).

Treatments	Number of fish stocked (fish/ unit)	Number of marketable fish produced* (fish/ unit)	Marketable fish (%)	
			A*	B
TLF	600	518.65	93.79 ^{b}	86.44 ^{b}
PLF	17895	16655	97.45 ^{a}	93.07 ^{a}
TPM	600	0	0 ^{e}	0 ^{e}
PPM	17895	10174.87	65.56 ^{c}	56.86 ^{c}
TCD	600	0	0 ^{e}	0 ^{e}
PCD	17895	818.45	5.61 ^{d}	4.57 ^{d}
TC	600	0	0 ^{e}	0 ^{e}
PC	17895	0	0 ^{e}	0 ^{e}

* Excluding deformed fish.

4.6. Studies on the effect of live-food treatment on fish production against conventional manuring regimen. (C) Examination of food selection and food preference of cultured fish in the different treatments (Experiment No. 6)

4.6.1. Organic manure quality

The amount of total nitrogen in the cow and poultry manures was 2.02% and 2.79%, respectively, whereas, the amount of organic carbon was 23.19% and 31.74%, respectively.

4.6.2. Water quality

Water temperature was between 27°C and 36°C during the 11 weeks. However, there was no difference in water temperature between the treatments on any given sampling day. The pH ranged from 5.2 to 8.0 (**Table 26**). Values of DO were significantly higher ($P < 0.05$) in the LF treatment than in PM or CD treatments. Average specific conductivity and NO_2 - N were significantly higher ($P < 0.05$) in PM, compared to other treatments. Total alkalinity here refers to HCO_3 alkalinity as CO_3 was absent in any of the treatments during the study period. The values of alkalinity, BOD, NH_4 - N, NO_3 - N and PO_4 - P were significantly higher ($P < 0.05$) in the manured treatments (PM and CD), compared to LF and C treatments (**Table 26**).

4.6.3. Plankton abundance (in environment and fish gut)

The composition of planktonic food organisms as observed in the environment and gut content of koi carp larvae held in the four management regimes are presented in **Table 27**. The cladocerans were relatively higher in diet abundance in the LF treatment with a mean value of 82.14% in the gut and 63.89% in the environment, compared to copepods (17.85% and 29.69%, respectively). The contribution of cladocerans was lower in the other treatments relative to LF (**Table 27**), although in the cow dung treated ponds (CD), the abundance of cladocerans in fish gut (44.71%) was higher than copepods (40.12%). *Moina* was the most dominant cladoceran in all the treatments ranging from 5% (C) to 27.95% (LF) in the environment, and 6.34% (C) to 31.81% (LF) in carp gut.

The copepods, on the other hand were relatively more abundant in the environment of control and manured ponds with values ranging from 48.71% in CD to 61.33% in C. *Cyclops* was the most dominant copepod in all the treatments (**Table 27**). Average plankton volume (no./ L) was highest in the LF treatment, followed in decreasing order by PM, CD and C treatments ($P < 0.55$; **Figure 3**).

The results of the electivity index (**Table 27**) estimate showed that koi carp larvae had a positive electivity towards cladocerans in all the treatments. Electivity towards copepods was however generally negative, except treatment C, where the result (+ 0.005) was insignificant and indicated absence of any food selection. Negative electivity for rotifers and phytoplankton was also observed in all the treatments with values ranging from - 0.181 (PM) to - 1.0 (LF) for rotifers and - 0.075 (CD) to - 1.0 (LF) for phytoplankton (**Table 27**).

4.6.4. Fish growth and survival

At harvest, maximum weight gain of koi carp (**Table 28**) was achieved in the LF treatment, followed in decreasing order by the PM, CD and C treatments ($P < 0.05$). SGR was also significantly higher ($P < 0.05$) in LF (5.45%/ day), compared to other treatments. There was a significant difference ($P < 0.05$) in survival of koi carp among the different treatments, ranging from 70.60% (C) to 96.16% (LF). The number of deformed koi carp was highest ($P < 0.05$) in the C treatment (**Table 28**).

4.6.5. Number of marketable fish

To determine the output of marketable fish, the percentage and number of fish above a total weight of 4 g (excluding deformed individuals) was estimated from the probability distribution at the end of the study (**Table 29**). The output of marketable fish was highest in the LF treatment (**Table 29**), followed in decreasing order by the PM, CD, and C treatments ($P < 0.05$).

Table 26 Mean ± SE of major water quality parameters analyzed for the four treatments. Each mean value represents 12 samples collected at weekly intervals during the 11-week growth period (3 March – 19 May, 2004). Different superscripts in the same row indicate statistically significant differences between means at $P < 0.05$. For pH, the range of recorded values are presented.

Parameters	Treatments			
	LF	PM	CD	C
pH	6.9 – 8.0	5.8 – 7.6	5.2 – 7.5	6.5 – 7.8
Dissolved oxygen (mg/ L)	7.32 ± 0.10^{a}	5.19 ± 0.23^{b}	5.67 ± 0.18^{b}	6.97 ± 0.13^{a}
BOD (mg/ L)	1.67 ± 0.06^{c}	2.61 ± 0.14^{a}	2.18 ± 0.10^{ab}	1.82 ± 0.08^{bc}
Free CO_2 (mg/ L)	2.57 ± 0.10^{c}	3.47 ± 0.12^{a}	3.18 ± 0.16^{ab}	2.84 ± 0.13^{bc}
Total alkalinity (mg/ L)	31.75 ± 1.18^{b}	79.92 ± 5.14^{a}	70.25 ± 4.28^{a}	34.42 ± 1.86^{b}
PO_4 – P (mg/ L)	0.23 ± 0.021^{b}	0.53 ± 0.059^{a}	0.45 ± 0.039^{a}	0.28 ± 0.024^{b}
NH_4 – N (mg/ L)	0.151 ± 0.014^{b}	0.332 ± 0.032^{a}	0.273 ± 0.024^{a}	0.295 ± 0.027^{a}
NO_2 – N (mg/ L)	0.009 ± 0.001^{c}	0.034 ± 0.003^{a}	0.021 ± 0.002^{b}	0.012 ± 0.001^{c}
NO_3 – N (mg/ L)	0.164 ± 0.014^{b}	0.412 ± 0.045^{a}	0.343 ± 0.032^{a}	0.19 ± 0.016^{b}
Specific conductivity (mmhos/ cm)	0.26 ± 0.016^{c}	0.64 ± 0.041^{a}	0.46 ± 0.026^{b}	0.27 ± 0.012^{c}

Table 27 Ivlev's Electivity Index applied to the gut contents of koi carp larvae along with percentage of plantonic organisms in fish gut and environment of the four management regimes.

	LF			PM			CD			C		
	% in Gut	% in Plankton	Ivlev's Index	% in Gut	% in Plankton	Ivlev's Index	% in Gut	% in Plankton	Ivlev's Index	% in Gut	% in Plankton	Ivlev's Index
Daphnia sp.	22.25	14.72	0.204	12.72	7.36	0.267	14.08	7.31	0.316	4.94	3.06	0.235
Moina sp.	31.81	27.95	0.065	18.13	11.69	0.216	18.24	11.93	0.209	6.34	5.00	0.118
Ceriodaphnia sp.	25.26	19.61	0.126	10.15	7.01	0.183	10.52	7.75	0.152	4.28	3.62	0.084
Bosmina sp.	2.91	1.59	0.293	1.28	1.32	-0.015	1.86	1.89	-0.008	4.51	3.03	0.196
Cladocera	82.14	63.89	0.125	42.29	27.41	0.213	44.71	28.90	0.215	20.08	14.73	0.154
Cyclops sp.	11.89	16.67	-0.167	21.62	24.84	-0.069	18.78	23.49	-0.111	32.26	31.80	0.007
Diaptomus sp.	-	0.90	-1.0	6.90	7.63	-0.050	4.19	6.48	-0.215	11.56	11.49	0.003
Nauplii	5.96	12.11	-0.340	17.59	18.84	-0.034	17.14	18.72	-0.044	18.19	17.93	0.007
Copepoda	17.85	29.69	-0.249	46.12	51.32	-0.053	40.12	48.71	-0.097	62.02	61.33	0.005
Brachionus sp.	-	1.60	-1.0	3.51	4.87	-0.162	2.29	4.69	-0.344	3.92	5.04	-0.125
Keratella sp.	-	0.67	-1.0	2.63	3.97	-0.203	0.87	3.75	-0.623	2.19	4.16	-0.310
Rotifera	-	2.29	-1.0	6.14	8.85	-0.181	3.16	8.44	-0.455	6.12	9.21	-0.201
Chlorella sp.	-	0.81	-1.0	0.59	2.25	-0.585	1.82	2.63	-0.182	2.51	3.76	-0.199
Navicula sp.	-	1.91	-1.0	2.32	4.75	-0.344	4.92	5.47	-0.053	4.28	5.14	-0.091
Spirogyra sp.	-	0.63	-1.0	1.02	1.76	-0.266	1.60	1.71	-0.033	2.05	2.65	-0.128
Scenedesmus sp.	-	0.04	-1.0	-	0.25	-1.0	-	0.46	-1.0	-	0.30	-1.0
Phacus sp.	-	0.58	-1.0	1.50	3.03	-0.338	3.65	3.18	0.069	2.93	2.03	0.181
Synedra sp.	-	0.12	-1.0	-	0.33	-1.0	-	0.46	-1.0	-	0.82	-1.0
Phytoplankton	-	4.12	-1.0	5.44	12.42	-0.391	12.00	13.94	-0.075	11.77	14.73	-0.112

Table 28 Growth parameters recorded for koi carp reared in earthen ponds under different management regimes for 11 weeks (3 March – 19 May, 2004). Different superscripts in the same row indicate statistically significant differences ($P < 0.05$).

	Treatments			
Parameters	**LF**	**PM**	**CD**	**C**
Harvest weight (g)	8.67 ± 0.16^{a}	6.23 ± 0.18^{b}	4.37 ± 0.12^{c}	3.56 ± 0.11^{d}
Weight gain (g)	8.54 ± 0.16^{a}	6.10 ± 0.18^{b}	4.24 ± 0.12^{c}	3.43 ± 0.11^{d}
SGR (%/ day)	5.45 ± 0.14^{a}	5.03 ± 0.10^{b}	4.56 ± 0.07^{c}	4.30 ± 0.07^{d}
Deformed individuals (%)	1.9^{d}	10.05^{b}	5.57^{c}	18.07^{a}
Survival rate (%)	96.16^{a}	90.5^{b}	81.86^{c}	70.60^{d}

Table 29 Average number of marketable fish (those heavier than 4.0 g) produced*, together with marketable fish produced expressed as a percentage of total number of fish produced* (A) and as a percentage of number of fish stocked (B) in the four treatments. Different superscripts in a column indicate statistically significant differences ($P < 0.05$).

Treatments	Number of marketable fish produced* (fish/ pond)	Marketable fish (%)	
		A*	B
LF	16868 [a]	98.02 [a]	94.26 [a]
PM	14397 [b]	88.90 [b]	80.45 [b]
CD	12595.92 [c]	85.98 [c]	70.39 [c]
C	313.74 [d]	3.34 [d]	2.02 [d]

* Excluding deformed fish.

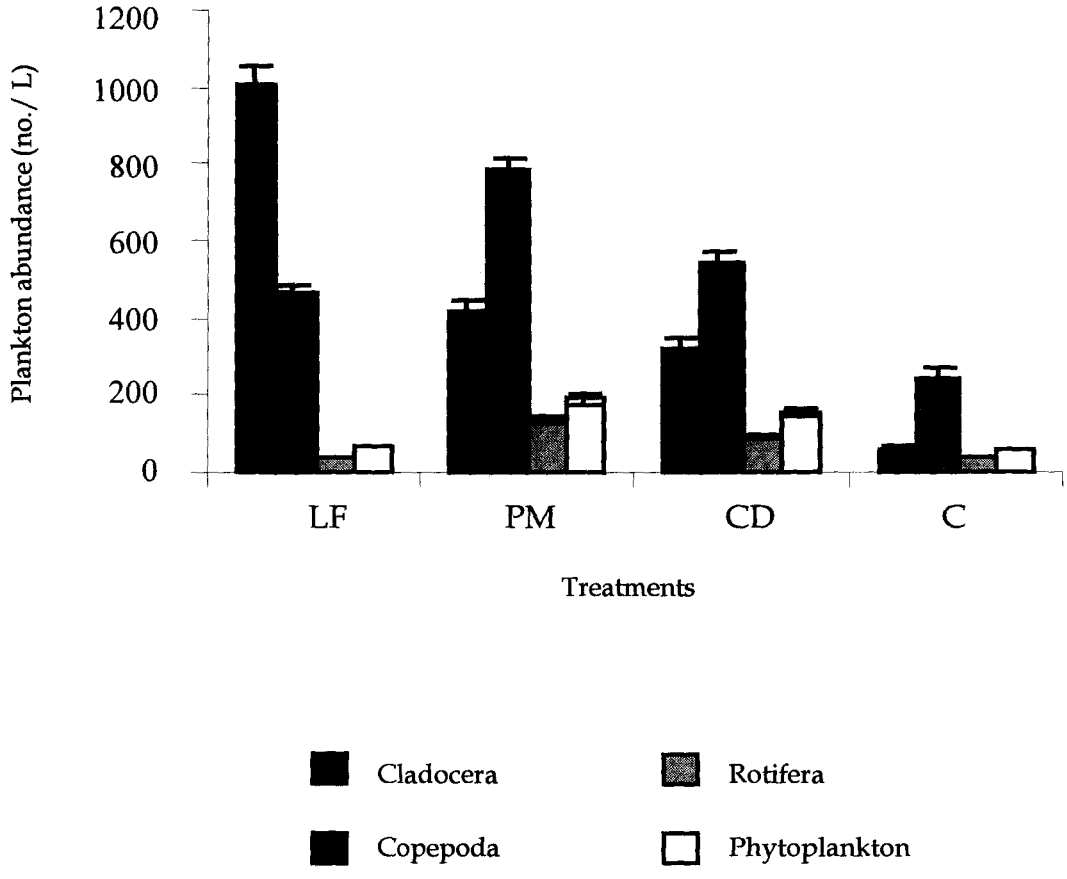

Figure 3 Average number of plankton in the four treatments.

4.7. Studies on the effect of live-food treatment on fish production against conventional manuring regimen. (D) Estimation of bacteriological counts of water and bottom sediment in the different treatments (Experiment No. 7)

4.7.1. Organic manure quality

The amount of total nitrogen in the cow and poultry manures was 2.12% and 2.59%, respectively, whereas, the amount of organic carbon was 22.06% and 28.52%, respectively.

4.7.2. Water and sediment quality

Water temperature was between 22°C and 38°C during the 11 weeks. There was no difference in water temperature between the different treatments on any given sampling day. The water pH in all the treatments was neutral to acidic (**Table 30**). The values of free CO_2 and total alkalinity were significantly higher in PM ($P < 0.05$), compared to the other treatments. Total alkalinity here refers to HCO_3 alkalinity, as CO_3 was not present in the water of any management regime during the entire study period. Average NH_4 - N, NO_2 - N, NO_3 - N, PO_4 - P, specific conductivity, and BOD were significantly higher ($P < 0.05$) in PM and CD, compared to the live-food (LF) and control treatments (**Table 30**). However, the values of DO and pH were significantly higher ($P < 0.05$) in the LF treatment, than other treatments (**Table 30**). The range of sediment pH was higher in LF and C, compared to the manured treatments (**Table 30**). The percentage of organic carbon and total nitrogen in the pond sediments were highest in the PM treatment ($P < 0.05$), followed by the CD, C, and LF treatments, although the values in the later two treatments did not record significantly differently ($P > 0.05$) from one another (**Table 30**).

4.7.3. Plankton abundance

Examination of plankton showed considerable differences in species diversity and abundance between different treatments. The cladocerans formed the most abundant group in LF, whereas copepods were more dominant in all the other treatments

(**Table 31**). On an average, total plankton density (no./ L) was highest in LF, followed by PM, CD, and C treatments in decreasing order ($P < 0.05$). Plankton population in all the treatments was dominated by zooplankton. Average zooplankton abundance (no./ L) also followed the same trend as the total plankton abundance and recorded highest in LF ($P < 0.05$) (**Table 31**). In contrast, average phytoplankton abundance (no./ L) was significantly higher ($P < 0.05$) in the manured treatments (PM and CD), compared to LF and C (**Table 31**).

4.7.4. Bacterial counts

Enumeration of heterotrophic bacterial population showed a highly variable result among the water of the four treatments. The average counts of heterotrophic bacteria were significantly higher ($P < 0.05$) in PM (123.58 x 10^3 cfus./ mL) and CD (95.75 x 10^3 cfus./ mL), than the LF and C treatments (**Table 32**). A marked difference in the mean counts of *Aeromonas* sp. and *Pseudomonas* sp. was also observed among the treatments (**Table 32**). Highest counts for both genera were observed in the PM treatment, followed in decreasing order by the CD and C treatments ($P < 0.05$). However, *Aeromonas* and *Pseudomonas* were absent from the water of LF ponds. In the pond sediments, there were no significant differences in the total aerobic heterotrophic counts between different treatments ($P > 0.05$) (**Table 32**). However, the *Aeromonas* and *Pseudomonas* bacterial counts of the pond sediments followed similar trends as the pond water and the highest counts for both these genera were encountered in PM, followed in decreasing abundance by the CD, C, and LF treatments ($P < 0.05$).

4.7.5. Fish growth and survival

At harvest, maximum weight gain of koi carp (**Table 33**) was achieved in the LF treatment, followed in the decreasing order by PM, CD, and C treatments ($P < 0.05$). The SGR was quite high (> 4.0) in all the treatments, though the differences among the various treatments were significant ($P < 0.05$). There was a significant difference ($P < 0.05$) in the survival of koi carp among the treatments ranging from 67.21% (C) to 90.11% (LF). The percentage of deformed koi carp was significantly higher in C ($P < 0.05$), compared to other treatments (**Table 33**).

4.7.6. Number of marketable fish

To determine the output of marketable fish, the percentage and number of fish above a total weight of 4 g (excluding deformed individuals) was estimated from the probability distribution at the end of the study (**Table 34**). The output of marketable fish was highest in the LF treatment (**Table 34**), followed in decreasing order by the PM, CD, and C treatments. Interestingly, while all the fish produced in the LF and PM treatments could be marketed, only 0.06% of the harvested fish from the control ponds (C) could attain marketable size (**Table 34**).

Table 30 Mean ± SE of major physico-chemical parameters analyzed for water and bottom sediments of the four treatments. Each value represents 12 samples collected at weekly intervals during the 11-week growth period (2 June – 18 August, 2004). Different superscripts in the same row indicate statistically significant differences between means at $P < 0.05$. For pH, the range of recorded values are presented.

Parameters	Treatments			
	LF	PM	CD	C
		Water		
pH	6.3 – 7.7	5.3 – 6.6	5.6 – 6.8	6.1 – 7.5
Dissolved oxygen (mg/ L)	6.63 ± 0.28 ^{a}	5.45 ± 0.25 ^{b}	5.79 ± 0.23 ^{ab}	6.35 ± 0.30 ^{ab}
BOD (mg/ L)	1.65 ± 0.09 ^{b}	4.22 ± 0.47 ^{a}	3.30 ± 0.31 ^{a}	1.85 ± 0.15 ^{b}
Free CO_2 (mg/ L)	2.08 ± 0.10 ^{c}	4.88 ± 0.26 ^{a}	3.57 ± 0.23 ^{b}	2.37 ± 0.10 ^{c}
Total alkalinity (mg/ L)	24.67 ± 0.85 ^{c}	72.22 ± 4.27 ^{c}	58.17 ± 3.13 ^{b}	29.90 ± 0.97 ^{c}
PO_4 – P (mg/ L)	0.16 ± 0.010 ^{b}	0.69 ± 0.083 ^{a}	0.51 ± 0.059 ^{a}	0.21 ± 0.017 ^{b}
NH_4 – N (mg/ L)	0.161 ± 0.011 ^{b}	0.569 ± 0.064 ^{a}	0.514 ± 0.048 ^{a}	0.203 ± 0.014 ^{b}
NO_2 – N (mg/ L)	0.006 ± 0.001 ^{b}	0.047 ± 0.006 ^{a}	0.038 ± 0.004 ^{a}	0.007 ± 0.001 ^{b}
NO_3 – N (mg/ L)	0.121 ± 0.007 ^{b}	0.462 ± 0.053 ^{a}	0.380 ± 0.046 ^{a}	0.148 ± 0.013 ^{b}
Specific conductivity (mmhos/ cm)	0.305 ± 0.012 ^{b}	0.711 ± 0.078 ^{a}	0.606 ± 0.070 ^{a}	0.364 ± 0.025 ^{b}
		Soil		
pH	5.2 – 6.8	4.6 – 5.9	4.5 – 5.8	5.0 – 6.8
Organic C (%)	1.27 ^{c}	2.94 ^{a}	2.02 ^{b}	1.32 ^{c}
Total N (%)	0.093 ^{c}	0.260 ^{a}	0.215 ^{b}	0.116 ^{c}

Table 31 Species composition, abundance (no./ L) and relative abundance (% of total numbers) of plankton in experimental ponds maintained under different management regimes. Each mean value represents data from 22 samples collected two times a week during the 11-week growth period (2 June – 18 August, 2004).

Plankton	LF		PM		CD		C	
	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)	(no./ L)	(%)
Daphnia sp.	274.38	16.90	168.13	11.80	122.64	9.96	5.26	1.91
Moina sp.	306.34	18.87	198.70	13.95	133.06	10.81	15.11	5.48
Bosmina sp.	108.26	6.67	81.43	5.72	64.19	5.21	3.20	1.16
Cladocera	688.98	42.44	448.26	31.47	319.89	25.98	23.57	8.55
Cyclops sp.	281.65	17.35	261.91	18.39	254.20	20.64	72.77	26.41
Diaptomus sp.	262.80	16.19	235.28	16.52	208.05	16.89	59.10	21.45
Nauplii	86.14	5.30	105.38	7.40	95.49	7.75	42.34	15.36
Copepoda	630.59	38.84	602.57	42.31	557.74	45.29	174.21	63.22
Brachionus sp.	55.14	3.39	58.90	4.13	62.34	5.06	10.29	3.73
Keratella sp.	106.62	6.57	82.42	5.79	71.52	5.81	12.48	4.53
Rotifera	161.76	9.96	141.32	9.92	133.86	10.87	22.77	8.26
Chlorella sp.	56.91	3.51	48.32	3.39	46.19	3.75	17.23	6.25
Navicula sp.	58.13	3.58	72.38	5.08	61.95	5.03	20.69	7.51
Spirogyra sp.	3.71	0.23	38.15	2.68	42.21	3.43	5.61	2.03
Scenedesmus sp.	1.04	0.06	18.24	1.28	16.18	1.31	3.02	1.09
Phacus sp.	21.20	1.30	29.16	2.05	39.20	3.18	3.16	1.15
Synedra sp.	1.13	0.07	25.86	1.81	14.12	1.15	5.29	1.93
Phytoplankton	142.12	8.75	232.11	16.29	219.85	17.85	55.00	19.96
Total Plankton	1623.45	-	1424.26	-	1231.34	-	275.55	-

Table 32 Abundance of total heterotrophic bacteria, *Aeromonas* sp. and *Pseudomonas* sp. in the water and bottom sediment analyzed for the four treatments. Each mean value represents weekly data collected during the 11-week growth period (2 June – 18 August, 2004). Different superscripts in the same row indicate statistically significant differences between means at $P < 0.05$.

	Treatments			
	LF	**PM**	**CD**	**C**
		Water		
Total heterotrophic bacteria (cfus. x 10^3/ mL)	17.08 [b]	123.58 [a]	95.75 [a]	28.17 [b]
Aeromonas sp. (cfus./ mL)	–	3.63 [a]	2.01 [b]	1.08 [c]
Pseudomonas sp. (cfus./ mL)	–	2.10 [a]	1.29 [b]	+
		Sediment		
Total heterotrophic bacteria (cfus. x 10^5/ g)	32.42 [a]	38.12 [a]	36.28 [a]	38.10 [a]
Aeromonas sp. (cfus./ g)	+	13.88 [a]	10.21 [b]	4.28 [c]
Pseudomonas sp. (cfus./ g)	+	9.04 [a]	7.19 [b]	3.76 [c]

+ = < 1.0; – = absent

Table 33 Growth parameters recorded for koi carp reared in earthen ponds under different management regimes for 11 weeks (2 June – 18 August, 2004). Different superscripts in the same row indicate statistically significant differences ($P < 0.05$).

	Treatments			
	LF	**PM**	**CD**	**C**
Harvest weight (g)	9.64 ± 0.28^{a}	6.83 ± 0.21^{b}	4.17 ± 0.15^{c}	3.14 ± 0.18^{d}
Weight gain (g)	9.51 ± 0.28^{a}	6.70 ± 0.21^{b}	4.04 ± 0.15^{c}	3.01 ± 0.18^{d}
SGR (%/ day)	5.58 ± 0.14^{a}	5.14 ± 0.09^{b}	4.51 ± 0.10^{c}	4.13 ± 0.04^{d}
Deformed individuals (%)	2.75^{c}	8.00^{b}	7.96^{b}	14.13^{a}
Survival rate (%)	90.11^{a}	84.50^{a}	78.18^{b}	67.21^{c}

Table 34 Average number of marketable fish (those heavier than 4.0 g) produced*, together with marketable fish produced, expressed as a percentage of total number of fish produced* (A) and as a percentage of number of fish stocked (B) in the four treatments. Different superscripts in a column represent statistically significant differences ($P < 0.05$).

Treatments	Number of fish stocked (fish/ pond)	Number of marketable fish produced* (fish/ pond)	Marketable fish (%)	
			A*	B
LF	17895	15682 [a]	100 [a]	87.63 [a]
PM	17895	13912 [b]	100 [a]	77.74 [b]
CD	17895	8661.63 [c]	75.04 [b]	48.40 [c]
C	17895	6.57 [d]	0.06 [c]	0.04 [d]

* Excluding deformed fish.

4.8. Studies on experimental ornamental fish polyculture. (A) Behavioural responses of two popular ornamental carps, koi carp and goldfish to monoculture and polyculture conditions in aquaria (Experiment No. 8)

Under monoculture conditions, the two species tended to swim at different depths in absence of food in both the experimental batches, with goldfish preferring deeper waters, while koi carp occupied the middle tank level (**Figure 4**; **Figure 5**). When food was present, both species in the first batch tended to move closer to the food (tubifex worm) and occupied the bottom tank levels (**Figure 4**). However, in the second batch, when live zooplankton was administered in the tanks, monocultured koi carp moved closer to the surface (**Figure 5**), while the mean depth of monocultured goldfish was similar to the one recorded when food was absent.

Under polyculture conditions, the mean depth of goldfish in absence of food was similar to the mean depth recorded under monoculture conditions. However, the polycultured koi carp moved deeper in the tank and its mean depth increased significantly ($P < 0.05$), compared to its preferred depth in the absence of food under monoculture conditions (**Figure 4**; **Figure 5**). When zooplankton was applied in the aquaria (second batch), the polycultured koi carp recorded a mean depth of 7.22 cm, which was significantly different ($P < 0.05$) to the mean depth recorded for monocultured koi carp in presence of plankton food (33.71 cm) (**Figure 5**). However, in the first batch, the availability of food (tubifex worm) in the bottom level influenced the polycultured koi carp's preference for the bottom tank level (**Figure 4**). No shifts were observed in the mean depth recorded for goldfish between monoculture and polyculture conditions in the presence of food (**Figure 4**; **Figure 5**).

Attack levels as estimated from the standardized chasing and nipping frequency per trial for both species were significantly higher with combined treatments, compared to monoculture, both in absence and presence of food ($P < 0.05$). The attack level also increased markedly in the presence of food, compared to when food was absent in both experimental batches (**Table 35**). Total attacks per trial (chases plus nips) as estimated from the different species level interactions (**Table 35**) showed that goldfish received more attacks under polyculture conditions in both batches, compared to koi carp. Under monoculture conditions, the incidence of attacks in goldfish tanks were lower compared to koi carp tanks, indicating koi carp as the more aggressive species. Chasing (**Figure 6**; **Figure 7**) and nipping (**Figure 8**; **Figure 9**) frequencies followed similar trends, although chasing frequency was greater than nipping frequency (**Table 35**).

Table 35 Standardized frequency of attacks calculated for koi carp (K) and goldfish (G) of the two experimental batches. 'Total attacks' refer to chases plus nips. For each interaction under monoculture and polyculture conditions, the ratio of nips to chases, and the ratio of total attacks during the presence of food to total attacks during absence of food are also shown. (+) indicates an increase in the presence of food, but where a ratio cannot be calculated due to a zero 'no food' value.

Interactions	Total attacks/ trial	Nips: chases	Food: no food
	First Batch		
Monoculture			
K → K	1.258	0.426	2.778
G → G	0.544	0.424	3.945
Polyculture			
K → K	0.913	0.520	3.492
K → G	2.944	0.584	1.769
G → G	0.846	0.065	1.735
G → K	0.077	0.185	+
	Second Batch		
Monoculture			
K → K	0.845	0.335	1.807
G → G	0.294	0.556	2.379
Polyculture			
K → K	0.571	0.903	1.584
K → G	2.708	0.605	1.396
G → G	1.330	0.360	1.509
G → K	0.156	0.902	+

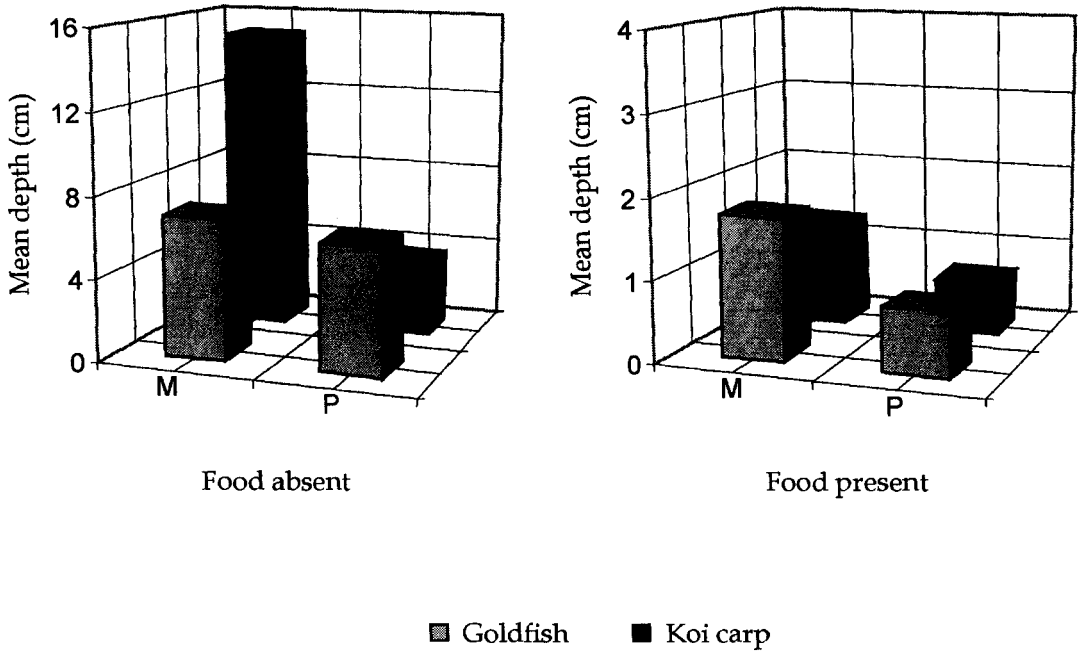

Figure 4 Mean depth recorded for koi carp and goldfish under monoculture (M) and polyculture (P) conditions in aquaria in the presence and absence of food (tubifex).

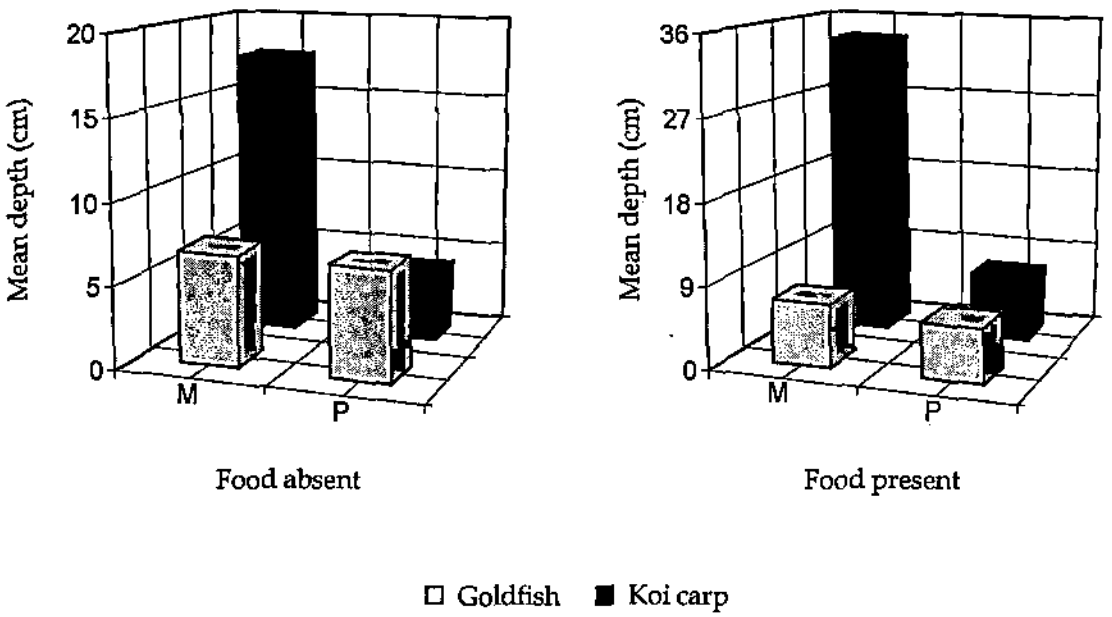

Figure 5 Mean depth recorded for koi carp and goldfish under monoculture (M) and polyculture (P) conditions in aquaria in the presence and absence of food (plankton).

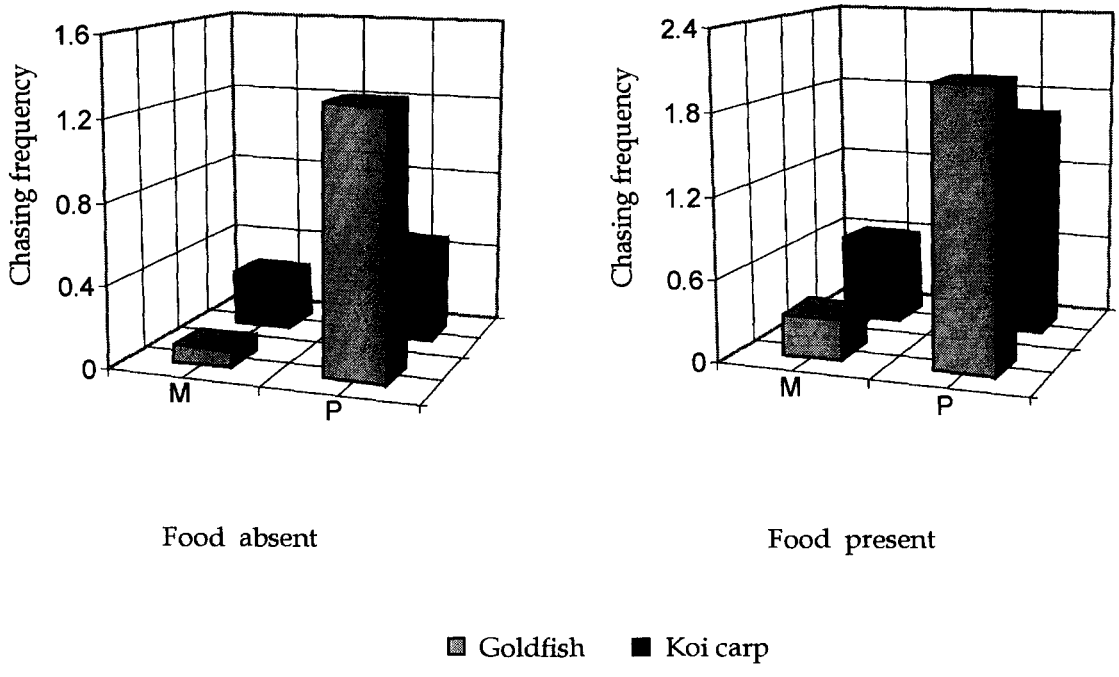

Figure 6 Standardized (per capita) chasing frequency per trial estimated for koi carp and goldfish under monoculture (M) and polyculture (P) conditions in aquaria in the presence and absence of food (tubifex).

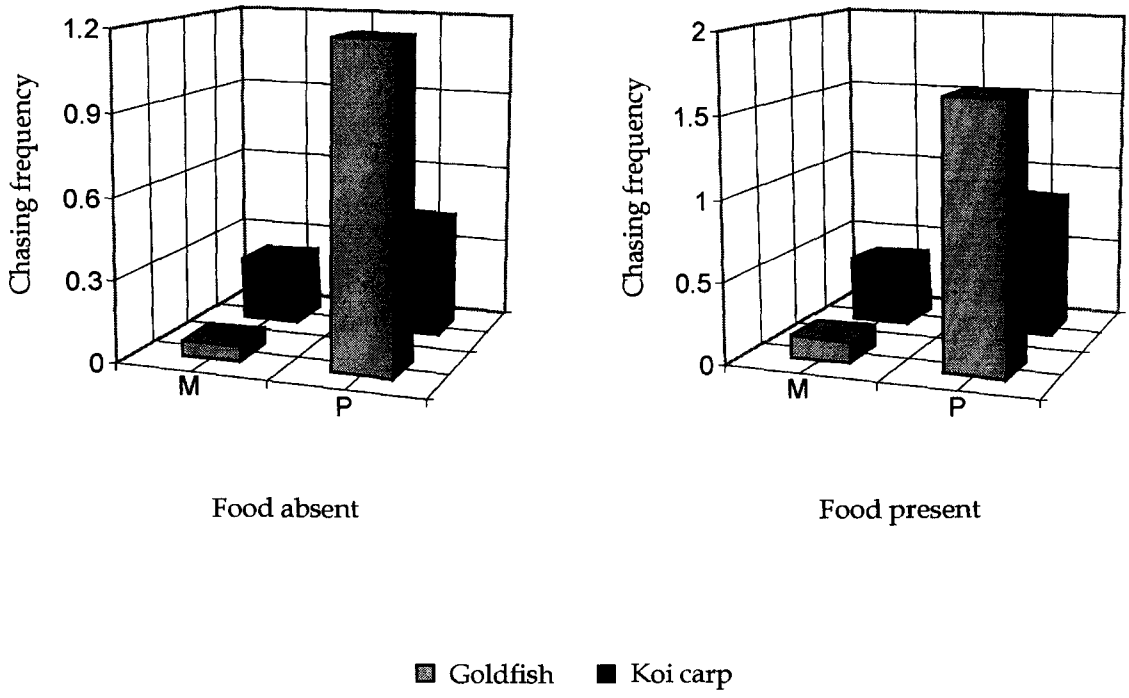

Figure 7 Standardized (per capita) chasing frequency per trial estimated for koi carp and goldfish under monoculture (M) and polyculture (P) conditions in aquaria in the presence and absence of food (plankton).

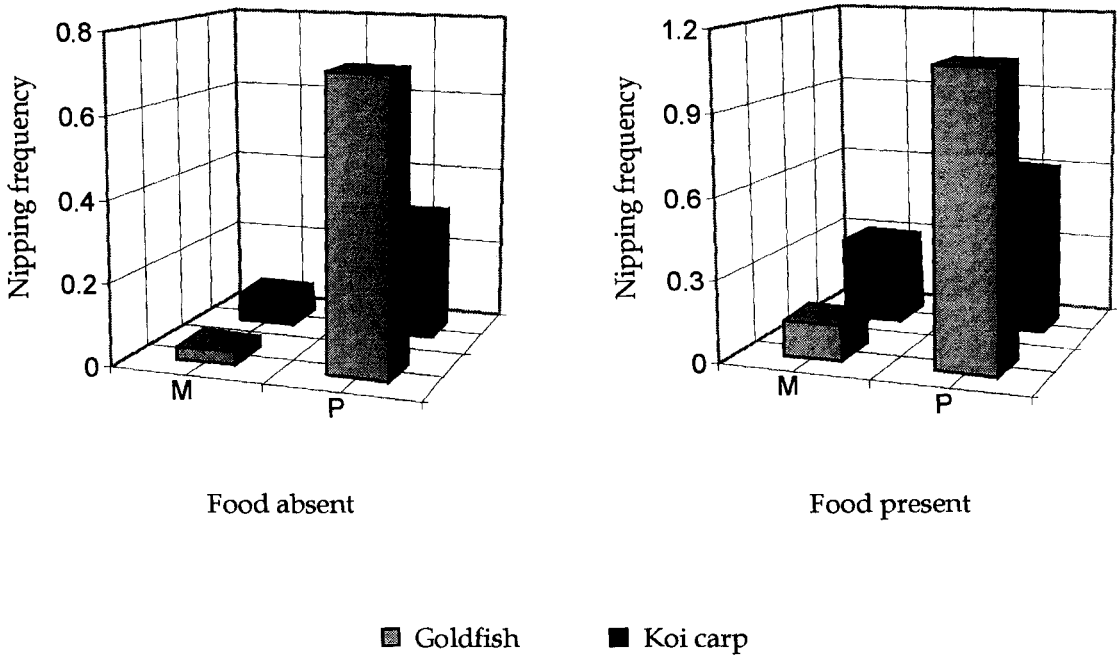

Figure 8 Standardized (per capita) nipping frequency per trial estimated for koi carp and goldfish under monoculture (M) and polyculture (P) conditions in aquaria in the presence and absence of food (tubifex).

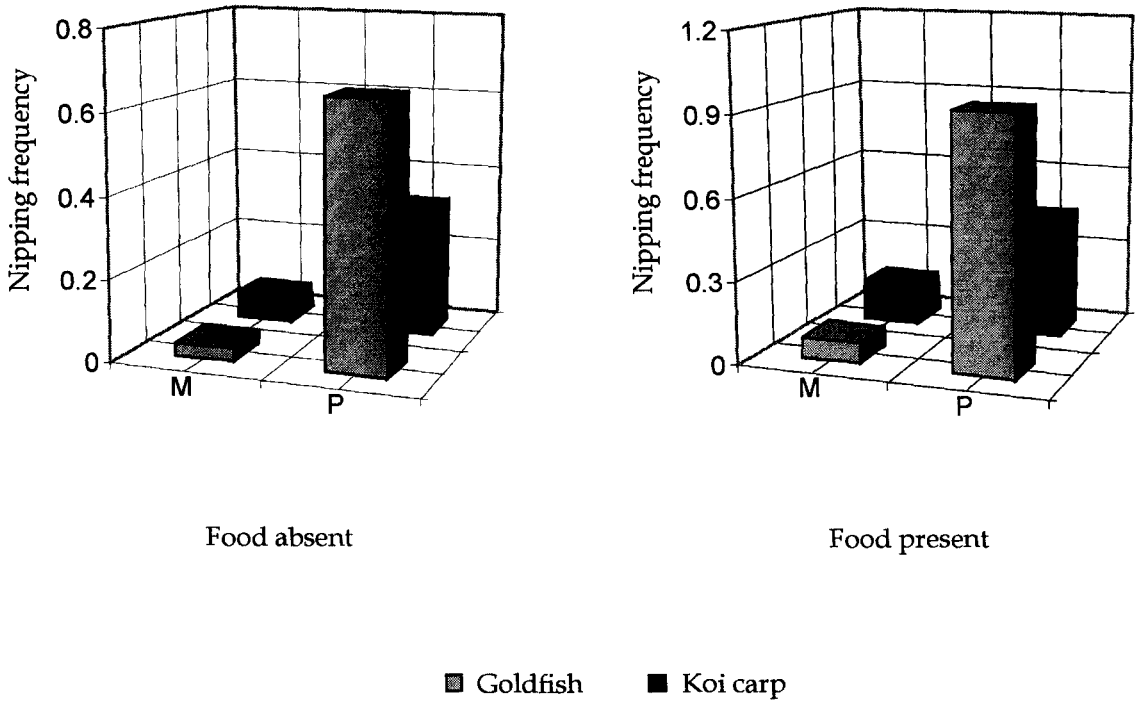

Figure 9 Standardized (per capita) nipping frequency per trial estimated for koi carp and goldfish under monoculture (M) and polyculture (P) conditions in aquaria in the presence and absence of food (plankton).

4.9. Studies on experimental ornamental fish polyculture. (B) Comparison of food selection and growth performance of koi carp and goldfish in monoculture and polyculture rearing in tropical ponds (Experiment No. 9)

4.9.1. Water quality

Water temperature was between 19°C and 30°C during the 11 weeks. However, there was no difference in the water temperature between the different treatments on any particular sampling day. The 100%K and 90%K-10%G treatments recorded significantly lower values of DO ($P < 0.05$), compared to other treatments (**Table 36**). The range of the recorded pH values was also lower in these two treatments. Significantly higher ($P < 0.05$) values of free CO_2 and total alkalinity were also recorded in 100%K and 90%K-10%G. Highest values of BOD and NO_3 - N were recorded in 100% K ($P < 0.05$). However, there were no significant differences in the values of specific conductivity, NO_2 - N, NH_4 - N and PO_4 - P, recorded in the different treatments ($P > 0.05$; **Table 36**).

4.9.2. Plankton abundance (in environment)

Average plankton composition in the environment of the experimental ponds is presented in **Table 37**. The plankton abundance and species diversity were similar in all the ponds, since they received daily plankton water from the same source. Cladocerans were in higher abundance in the environment (54.11%), compared to copepods (38.05%). Rotifers constituted 2.62% of total plankton composition, while phytoplankton was 5.21%. *Moina* and *Daphnia* were most abundant among cladocerans, while *Cyclops* was the most dominant copepod (**Table 37**).

4.9.3. Plankton abundance (in fish gut)

The compositions of planktonic food organisms in the gut contents of koi carp and goldfish are presented in **Table 38** and **Table 39**, respectively. In both species, cladocerans were relatively higher in diet abundance, ranging from 83.62% (100%K) to 87.04% (10%K-90%G) for koi carp (**Table 38**), and 66.62% (90%K-10%G) to 94.62% (100%G)

for goldfish (**Table 39**). The copepods, on the other hand, contributed only 12.84% (10%K-90%G) to 14.40% (100%K) in koi carp gut (**Table 38**), and 5.38% (100%G) to 29.93% (90%K-10%G) in goldfish gut (**Table 39**).

The results of the electivity index estimate revealed that both species of fish preferred cladocerans in all treatments. Electivity towards other zooplankton groups (copepods and rotifers) and phytoplankton were negative for both the carps. However, for goldfish, the values of the positive electivity towards cladocerans differed significantly from one treatment to another ($P < 0.05$), ranging from 0.104 (90%K-10%G) to 0.272 (100%G). Likewise, the values of the negative electivity towards the copepods also differed significantly ($P < 0.05$) among the goldfish stocked in the various treatments (**Table 39**). In sharp contrast, for the koi carp, the levels of the positive selection of cladocerans, or negative selection of copepods, as evidenced from the electivity indices (**Table 38**) did not differ significantly ($P > 0.05$) between the different treatments.

4.9.4. Fish growth and survival

At harvest, there were no significant differences in the weight gain, SGR and the percentage of deformed fish estimated for koi carp among the various treatments ($P > 0.05$; **Table 40**). The survival rate of koi carp ranged from 92.59% (30%K-70%G) to 95.70% (90%K-10%G). However, polyculture had a significant effect on the growth parameters of goldfish (**Table 40**) and the highest weight gain (9.36 g) was recorded in the monoculture treatment (100%G). The SGR was quite high in all the treatments (> 3.5), though the differences among the treatments were significant ($P < 0.05$). The survival rate of goldfish was significantly higher ($P < 0.05$) in the monoculture treatment (91.41%), compared with the different polyculture treatments (**Table 40**). The percentage of goldfish deformities was highest in 90%K-10%G, followed in decreasing order by 70%K-30%G, 50%K-50%G, 30%K-70%G, 10%K-90%G and 100%G treatments ($P < 0.05$; **Table 40**).

4.9.5. Number of marketable fish

To determine the output of marketable fish, the percentage and number of fish above a total weight of 4 g (excluding deformed individuals) was estimated from the probability distribution at the end of the study (**Table 41**). Polyculture had a direct

effect on fish production since the monoculture treatments for each species produced the highest percentage of marketable fish (**Table 41**). Among the various polyculture treatments, 90%K-10%G and 10%K-90%G produced a significantly greater number and percentage of marketable fish ($P < 0.05$), compared to other treatments (**Table 41**). All the fish harvested in the 100%K, 100%G and 10%K-90%G treatments (excluding deformed fish) attained marketable size (**Table 41**).

Table 36 Mean ± SE of major water quality parameters analyzed for the seven treatments. Each mean value represents 12 samples collected at weekly intervals during the 11-week growth period (5 September – 18 November, 2004). Different superscripts in the same row indicate statistically significant differences between means at $P < 0.05$. For pH, the range of recorded values are presented.

Parameters	Treatments						
	100%K	90%K – 10%G	70%K – 30%G	50%K – 50%G	30%K – 70%G	10%K – 90%G	100%G
pH	5.5 – 7.4	5.5 – 7.4	6.0 – 8.1	6.5 – 7.9	6.4 – 8.1	6.4 – 8.0	6.6 – 7.8
Dissolved oxygen (mg/ L)	5.75 ± 0.28 ^{b}	5.72 ± 0.09 ^{b}	5.92 ± 0.14 ^{ab}	6.27 ± 0.31 ^{a}	6.20 ± 0.16 ^{a}	6.24 ± 0.19 ^{a}	6.31 ± 0.28 ^{a}
Free CO_2 (mg/ L)	2.92 ± 0.09 ^{a}	2.86 ± 0.14 ^{a}	2.60 ± 0.08 ^{b}	2.51 ± 0.12 ^{b}	2.58 ± 0.11 ^{b}	2.62 ± 0.16 ^{b}	2.59 ± 0.14 ^{b}
BOD (mg/ L)	1.31 ± 0.04 ^{a}	1.19 ± 0.08 ^{ab}	1.20 ± 0.06 ^{ab}	1.08 ± 0.05 ^{bc}	0.95 ± 0.10 ^{c}	1.05 ± 0.09 ^{c}	1.01 ± 0.05 ^{c}
Total alkalinity (mg/ L)	38.26 ± 2.19 ^{a}	37.10 ± 1.87 ^{a}	30.04 ± 1.04 ^{bc}	28.19 ± 0.72 ^{c}	31.12 ± 1.29 ^{bc}	30.60 ± 0.88 ^{bc}	33.92 ± 1.40 ^{b}
PO_4 – P (mg/ L)	0.32 ± 0.041 ^{a}	0.32 ± 0.035 ^{a}	0.30 ± 0.031 ^{a}	0.29 ± 0.034 ^{a}	0.27 ± 0.028 ^{a}	0.29 ± 0.016 ^{a}	0.28 ± 0.028 ^{a}
NH_4 – N (mg/ L)	0.167 ± 0.030 ^{a}	0.165 ± 0.021 ^{a}	0.168 ± 0.014 ^{a}	0.152 ± 0.026 ^{a}	0.155 ± 0.029 ^{a}	0.152 ± 0.022 ^{a}	0.155 ± 0.021 ^{a}
NO_2 – N (mg/ L)	0.015 ± 0.004 ^{a}	0.015 ± 0.005 ^{a}	0.013 ± 0.001 ^{a}	0.012 ± 0.003 ^{a}	0.013 ± 0.002 ^{a}	0.012 ± 0.003 ^{a}	0.011 ± 0.002 ^{a}
NO_3 – N (mg/ L)	0.160 ± 0.018 ^{a}	0.142 ± 0.014 ^{b}	0.135 ± 0.015 ^{b}	0.116 ± 0.012 ^{c}	0.125 ± 0.013 ^{bc}	0.128 ± 0.011 ^{bc}	0.121 ± 0.012 ^{c}
Specific conductivity (mmhos/ cm)	0.26 ± 0.013 ^{a}	0.25 ± 0.012 ^{a}	0.23 ± 0.014 ^{a}	0.21 ± 0.029 ^{a}	0.23 ± 0.032 ^{a}	0.24 ± 0.014 ^{a}	0.23 ± 0.018 ^{a}

Table 37 Species composition, abundance (no./ L) and relative abundance (% of total numbers) of plankton present in the experimental ponds. Each mean value represents data from 22 samples collected two times a week during the 11-week growth period (5 September – 18 November, 2004).

Plankton	no./ L	%
Daphnia sp.	314.20	17.04
Moina sp.	389.15	21.11
Ceriodaphnia sp.	218.07	11.83
Bosmina sp.	76.17	4.13
Cladocera	997.59	54.11
Cyclops sp.	380.24	20.62
Diaptomus sp.	39.60	2.15
Nauplii	281.64	15.28
Copepoda	701.48	38.05
Brachionus sp.	28.92	1.57
Keratella sp.	19.39	1.05
Rotifera	48.31	2.62
Chlorella sp.	18.35	0.99
Navicula sp.	40.12	2.18
Spirogyra sp.	21.07	1.14
Scenedesmus sp.	1.32	0.07
Phacus sp.	14.10	0.77
Synedra sp.	1.19	0.06
Phytoplankton	96.15	5.21
Total Plankton	1843.53	–

Table 38 Ivlev's Electivity Index applied to the gut contents of koi carp larvae along with percentage of planktonic organisms in the fish gut.

Plankton	Treatments											
	100%K		90%K – 10%G		70%K – 30%G		50%K – 50%G		30%K – 70%G		10%K – 90%G	
	% in Gut	Ivlev's index	% in Gut	Ivlev's index	% in Gut	Ivlev's index	% in Gut	Ivlev's index	% in Gut	Ivlev's index	% in Gut	Ivlev's index
Daphnia sp.	28.29	0.248	30.14	0.278	29.70	0.271	31.22	0.294	30.18	0.278	30.51	0.283
Moina sp.	29.51	0.166	30.96	0.189	27.39	0.129	28.09	0.142	29.79	0.171	32.16	0.207
Ceriodaphnia sp.	16.98	0.179	15.29	0.125	17.51	0.194	14.52	0.102	17.53	0.194	12.32	0.021
Bosmina sp.	8.84	0.363	9.65	0.401	10.38	0.431	12.04	0.489	8.29	0.335	12.05	0.489
Cladocera	83.62	0.214	86.04	0.228	84.98	0.222	85.87	0.227	85.79	0.226	87.04	0.233
Cyclops sp.	9.13	-0.386	8.05	-0.438	10.16	-0.340	9.86	-0.353	8.68	-0.407	9.05	-0.390
Diaptomus sp.	1.08	-0.331	0.95	-0.387	0.82	-0.448	0.21	-0.822	0.11	-0.903	0.16	-0.861
Nauplii	4.19	-0.569	4.05	-0.581	3.34	-0.641	3.78	-0.603	4.85	-0.518	3.63	-0.616
Copepoda	14.40	-0.451	13.05	-0.489	14.32	-0.453	13.85	-0.466	14.04	-0.461	12.84	-0.495
Brachionus sp.	0.28	-0.697	0.11	-0.869	0.17	-0.805	0.10	-0.880	-	-1	-	-1
Keratella sp.	0.65	-0.235	0.51	-0.346	0.34	-0.511	0.12	-0.795	0.07	-0.875	0.04	-0.927
Rotifera	0.93	-0.476	0.62	-0.617	0.51	-0.674	0.22	-0.845	0.07	-0.948	0.04	-0.970
Chlorella sp.	0.14	-0.752	-	-1	-	-1	-	-1	-	-1	-	-1
Navicula sp.	0.50	-0.627	0.21	-0.824	0.14	-0.879	0.06	-0.946	0.10	-0.912	0.08	-0.929
Spirogyra sp.	0.32	-0.562	0.08	-0.869	0.05	-0.916	-	-1	-	-1	-	-1
Scenedesmus sp.	-	-1	-	-1	-	-1	-	-1	-	-1	-	-1
Phacus sp.	0.09	-0.791	-	-1	-	-1	-	-1	-	-1	-	-1
Synedra sp.	-	-1	-	-1	-	-1	-	-1	-	-1	-	-1
Phytoplankton	1.05	-0.665	0.29	-0.894	0.19	-0.929	0.06	-0.977	0.10	-0.962	0.08	-0.969

Table 39 Ivlev's Electivity Index applied to the gut contents of goldfish larvae along with percentage of planktonic organisms in the fish gut.

Plankton	Treatments											
	90%K – 10%G		70%K – 30%G		50%K – 50%G		30%K – 70%G		10%K – 90%G		100%G	
	% in Gut	Ivlev's index	% in Gut	Ivlev's index	% in Gut	Ivlev's index	% in Gut	Ivlev's index	% in Gut	Ivlev's index	% in Gut	Ivlev's index
Daphnia sp.	19.04	0.055	19.18	0.059	20.01	0.080	22.31	0.134	27.02	0.226	35.10	0.346
Moina sp.	20.12	-0.024	20.56	-0.013	21.86	0.017	22.78	0.038	23.37	0.051	28.25	0.143
Ceriodaphnia sp.	15.01	0.118	14.38	0.113	15.47	0.133	17.94	0.205	16.08	0.152	15.10	0.121
Bosmina sp.	12.45	0.502	13.06	0.519	12.69	0.509	14.23	0.550	15.18	0.572	16.17	0.593
Cladocera	66.62	0.104	67.18	0.108	70.03	0.128	77.26	0.176	81.65	0.203	94.62	0.272
Cyclops sp.	16.27	-0.118	15.70	-0.135	15.21	-0.151	12.37	-0.250	10.14	-0.341	2.08	-0.817
Diaptomus sp.	2.05	-0.024	2.34	-0.066	1.85	-0.075	1.82	-0.083	1.38	-0.218	0.18	-0.845
Nauplii	11.61	-0.136	11.29	-0.150	10.12	-0.203	7.55	-0.338	6.32	-0.415	3.12	-0.661
Copepoda	29.93	-0.119	29.33	-0.129	27.18	-0.166	21.74	-0.273	17.84	-0.362	5.38	-0.752
Brachionus sp.	0.64	-0.421	0.65	-0.414	0.38	-0.610	0.28	-0.697	0.14	-0.836	-	-1
Keratella sp.	0.75	-0.166	0.68	-0.214	0.70	-0.200	0.26	-0.603	0.18	-0.707	-	-1
Rotifera	1.39	-0.307	1.33	-0.327	1.08	-0.416	0.54	-0.658	0.32	-0.782	-	-1
Chlorella sp.	0.30	-0.535	0.35	-0.478	0.31	-0.523	0.05	-0.904	-	-1	-	-1
Navicula sp.	0.81	-0.458	0.88	-0.425	0.63	-0.552	0.19	-0.840	0.09	-0.921	-	-1
Spirogyra sp.	0.74	-0.213	0.60	-0.310	0.57	-0.333	0.06	-0.900	-	-1	-	-1
Scenedesmus sp.	-	-1	-	-1	-	-1	-	-1	-	-1	-	-1
Phacus sp.	0.21	-0.571	0.33	-0.400	0.20	-0.588	0.16	-0.656	0.10	-0.770	-	-1
Synedra sp.	-	-1	-	-1	-	-1	-	-1	-	-1	-	-1
Phytoplankton	2.06	-0.433	2.16	-0.414	1.71	-0.506	0.46	-0.838	0.19	-0.930	-	-1

Table 40 Growth parameters recorded for koi carp and goldfish after mono and polyculture rearing for 11 weeks (5 September – 18 November, 2004). Different superscripts in the same row indicate statistically significant differences ($P < 0.05$).

	Treatments						
	100%K	**90%K – 10%G**	**70%K – 30%G**	**50%K – 50%G**	**30%K – 70%G**	**10%K – 90%G**	**100%G**
				koi carp			
Final weight (g)	8.05 ± 0.09^{a}	8.09 ± 0.18^{a}	7.95 ± 0.12^{a}	7.90 ± 0.18^{a}	7.90 ± 0.31^{a}	7.88 ± 0.14^{a}	-
Mean growth increment (g)	7.93 ± 0.09^{a}	7.97 ± 0.18^{a}	7.83 ± 0.12^{a}	7.78 ± 0.18^{a}	7.78 ± 0.31^{a}	7.76 ± 0.14^{a}	-
SGR (%/ day)	5.45 ± 0.09^{a}	5.47 ± 0.18^{a}	5.44 ± 0.12^{a}	5.44 ± 0.18^{a}	5.44 ± 0.31^{a}	5.43 ± 0.14^{a}	-
Survival (%)	95.50^{a}	95.70^{a}	94.44^{ab}	94.11^{ab}	92.59^{b}	92.73^{b}	-
Deformed individuals (%)	1.91^{a}	2.02^{a}	1.73^{a}	2.04^{a}	2.26^{a}	1.78^{a}	-
				goldfish			
Final weight (g)	-	3.65 ± 0.21^{c}	3.80 ± 0.24^{c}	3.87 ± 0.22^{c}	4.01 ± 0.28^{c}	6.44 ± 0.22^{b}	9.53 ± 0.3^{a}
Mean growth increment (g)	-	3.48 ± 0.21^{c}	3.63 ± 0.24^{c}	3.70 ± 0.22^{c}	3.84 ± 0.28^{c}	6.27 ± 0.22^{b}	9.36 ± 0.3^{a}
SGR (%/ day)	-	3.97 ± 0.21^{e}	4.03 ± 0.24^{d}	4.05 ± 0.22^{d}	4.10 ± 0.28^{c}	4.71 ± 0.22^{b}	5.21 ± 0.3^{a}
Survival (%)	-	54.36^{f}	60.70^{e}	65.51^{d}	71.90^{c}	81.07^{b}	91.4^{a}
Deformed individuals (%)	-	11.53^{a}	8.93^{b}	7.07^{c}	4.23^{d}	3.17^{de}	2.42^{e}

Table 41 The average number of marketable koi carp (K) and goldfish (G) (those heavier than 4.0 g) produced*, together with marketable fish produced expressed as a percentage of total number of fish produced* (A) and as a percentage of number of fish stocked (B) in the different treatments. Different superscripts in a column represent statistically significant differences ($P < 0.05$).

Treatments	Number of fish stocked (fish/ pond)			Number of marketable fish produced* (fish/ pond)			Marketable fish (%)	
	K	G	Total	K	G	Total	(A)*	(B)
100%K	17880	-	17880	16735	-	16735 [a]	100 [a]	94.0 [a]
90%K – 10%G	16092	1788	17880	15078	36.61	15114.61 [ab]	95.16 [b]	84.53 [c]
70%K – 30%G	12516	5364	17880	11602	561.87	12163.87 [c]	84.59 [c]	68.03 [e]
50%K – 50%G	8940	8940	17880	8231	1448.57	9679.57 [d]	71.94 [d]	54.14 [f]
30%K – 70%G	5364	12516	17880	4846	4355.66	9201.66 [d]	69.10 [e]	51.46 [g]
10%K – 90%G	1788	16092	17880	1626	12536	14162 [bc]	100 [a]	79.21 [d]
100%G	-	17880	17880	-	15909	15909 [ab]	100 [a]	88.98 [b]

* Excluding deformed fish.

5. Discussions

5.1. Studies on the effect of manuring rate on fish production (Experiment No. 1)

There was autochthonous production of plankton in all the treatments, following the principle of pond fertilization. Plankton formed the main source of food since there was a direct correlation ($r = 0.957$; $P < 0.01$) between the weight gain of koi carp and the amount of plankton present in the five treatments (**Figure 10**). Earlier studies on the common carp are indicative of their preference for plankton as food (Lubzens *et al.*, 1984; Chakrabarti and Jana, 1990).

High application rates of cow dung and poultry manure in the C3 and P3 treatments significantly increased ($P < 0.05$) the alkalinity, PO_4 - P, NH_4 - N, NO_2 - N and NO_3 - N values of water. NH_4 - N, incorporated from organic manure application, as well as metabolism of the water body, might be considered an index of environmental stress (Jana and Chakrabarti, 1993). Jana and Barat (1984) observed an inverse relationship between NH_4 - N and DO. In our experiment, lower DO values were recorded in the C3 and P3 treatments. Critical evaluation of the data revealed that the concentration of NH_4 - N was inversely related to the abundance of cladocerans in the C3 ($r = - 0.614$; $P < 0.05$) and P3 ($r = - 0.688$; $P < 0.05$) treatments. Differences in the relative abundance of some groups of zooplankton might have contributed to the differential growth responses of the fish. Lower weight gain, SGR and survival rate of koi carp in the control treatment may be attributed to the insufficient quantity of zooplankton in the system (Szlaminska and Przybye, 1986).

It is well known that high yield of fish can be achieved by greater abundance of plankton in the culture system. However, it is not possible to increase the application rate of organic manures after a certain limit because this may reduce water quality, which cause stress for reproduction of essential zooplankton thereby causing adverse effect on fish growth. Studies on life history parameters of *Daphnia* sp. (Jana and Pal, 1983; Jana and Pal, 1985 a; Murugan, 1989; Urabe, 1988; Urabe and Watanabe, 1992) and *Moina* sp. (Jana and Pal, 1985 b; Jana and Pal, 1989) suggest that growth, reproductive potentials and longevity of each species are affected by the nutrient conditions of culture media. Dhawan and Kaur (2002 a) reported a decrease in cladoceran population with increased organic manure application in ponds. The presence of relatively higher density

of zooplankton in C2 and P2, compared to C3 and P3 could be a consequence of relatively suitable environment in terms of water quality and food abundance (Jana and Chakrabarti, 1997). As a result, the weight gain of koi carp was significantly higher ($P < 0.05$) in the C2 and P2 treatments, compared to the C3 and P3 treatments, respectively. Similar decline in plankton abundance due to undesirable water quality with very high amounts of fertilizers have been reported by many authors (Lin *et al.*, 1997; Garg and Bhatnagar, 2000; Azim *et al.*, 2001; Cheikyula *et al.*, 2001). Perhaps, the significantly higher level of nutrients and low dissolved oxygen in the C3 and P3 treatments lowered the grazing activity by carp in these two treatments, compared to the C2 and P2 treatments, respectively. Again, the differences in the weight gain of koi carp observed among the different treatments were not essentially due to changes in the water quality, since, growth in the C1 and P1 treatments were significantly lower than C2 and P2 treatments, respectively, despite having good water quality. It might well be that the weight gain was more directly related to differences in food concentrations, although the zooplankton density and water quality were closely related to each other.

In any given application rate, the poultry manure appeared to be more effective compared to cow dung, which is in agreement with earlier findings by Singh and Sharma (1999). Poultry manure is preferred worldwide because of its high level of nitrogen and phosphorus concentrations (Knud-Hansen *et al.*, 1991). Although phosphorus was not analyzed in this study, total nitrogen in poultry manure was higher than cow dung. Nitrogen input was shown to be the key nutrient variable related to plankton production, which in turn influenced fish yield (Knud-Hansen *et al.*, 1993). In the present investigation, an application rate of 0.26 kg/ m^3 every 10 days, appeared to be the most suitable for koi carp tanks manured with cow dung and poultry excreta. Higher application rates reduced water quality, depleted the plankton population and caused adverse impact on the growth of fish.

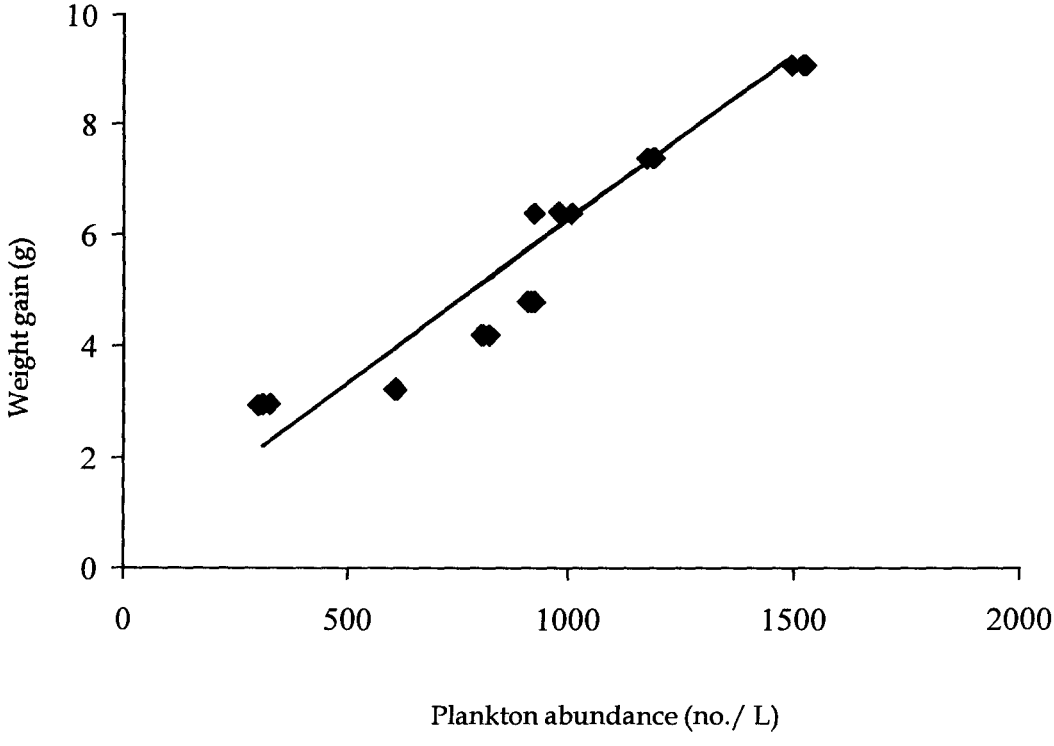

Figure 10 Relationship between weight gain of koi carp and plankton abundance in the seven treatments.

5.2. Studies on the effect of water exchange on fish production (Experiment No. 2)

In agreement with earlier findings (Chapter 5.1), there was autochthonous production of plankton in all the treatments, following the principal of pond fertilization. Plankton formed the main source of food since there was a direct correlation ($r = 0.986$; $P < 0.01$) between the weight gain of koi carp and the amount of plankton present in the five treatments (**Figure 11**).

Water exchange rates had a direct influence on the water quality in the different treatments. No exchange of water in the NE treatment significantly lowered the dissolved oxygen ($P < 0.05$) and simultaneously increased the specific conductivity, PO_4 - P, NH_4 - N, NO_2 - N, NO_3 - N and BOD, compared to the other treatments (**Table 8**). According to Pechar (2000), gradual accumulation of organic matter in a water body lead to subsequent dominance of biodegradation and decomposition processes and cause an oxygen deficit. The resulting release of nutrients leads to excessive levels of autotrophic production, as well as changes in the species composition of plankton. Through a dynamic multiple feed back process, the water quality deteriorates.

As discussed in Chapter 5.1, NH_4 - N might be considered an index of environmental stress. High concentrations of NH_4 - N were found to restrict the occurrence of many small protozoans like ciliates that are considered as excellent food for cladocerans (Pfister *et al.*, 2002). The presence of relatively higher density of cladocerans in the WE1, WE2, WE3 and WE4 treatments, compared to NE, might be a consequence of better environment in terms of water quality and food abundance. According to Herbert (1978), the maximum size reached by individuals of a particular species of *Daphnia* depends upon the food supply. Also, earlier studies on life history parameters of *Daphnia* sp. and *Moina* sp., as also discussed in Chapter 5.1, suggest that growth, reproductive potentials and longevity of each species are affected by the nutrient conditions of culture media.

Perhaps the significantly high level of nutrients and BOD, along with low DO concentration in the NE treatment lowered the grazing activity of the carp. The results clearly indicate that no water exchange in the NE treatment yielded the lowest number ($P < 0.05$) of saleable fish. In contrast to food fish production, where the total number of

fish produced determines productivity, ornamental fish can only be sold once they have reached a particular size. Systematic discharge of water in WE1, WE2, WE3 and WE4 treatments significantly increased ($P < 0.05$) the number of marketable fish. Greater dilution of the manure in these four treatments improved the water quality and caused greater abundance of plankton, compared to the NE treatment, though the plankton volume within the four water exchange regimes were significantly different ($P < 0.05$) from one another. Differences in the relative abundance of some group of zooplankton might have contributed to the differential growth responses and survival of the carp. Non availability or non continuous supply of preferred food have been reported to influence cannibalism in koi carp larvae (Appelbaum *et al.*, 1986; VanDamme *et al.*, 1989). Rothbard (1982) reported low survival rates in common carp as a result of severe competition for food when stocked at high densities. Interestingly, reduction in cannibalism in common carp was demonstrated by Von Lukowicz (1979), when a continuous supply of live food was maintained. Food availability is probably the most important factor determining the cannibalism rate in fish larvae (Hecht and Appelbaum, 1988). As observed in this experiment, the influence of plankton level on growth heterogeneity and survival rate of koi carp supports this last hypothesis.

Survival rate of the koi carp was also influenced by the water quality. Nitrite ions are toxic to fish, causing methaemoglobinemia (Tomasso *et al.*, 1979). It is present in water as an intermediate in the bacterial oxidation of ammonia, the major nitrogenous waste product of fish, to nitrate (Das *et al.*, 2004). An increase in the nitrite content in water exerts considerable stress on the fish resulting in growth suppression, tissue damage and mortality (Lewis and Morris, 1986), leading to poor biomass production. Diminished respiration ability in nitrite-exposed grass carp, *Ctenopharyngodon idella* was reported by Alcaraz and Espina (1997). Korwin-Kossakowski and Ostaszewska (2003) also reported on the adverse impact on respiration and growth of common carp due to nitrite exposure. Allowable levels are therefore low. NO_2 - N levels above 0.06 mg/ L has been observed to cause a minimal degree of harm in rainbow trout, *Oncorhynchus mykiss* after 3 weeks exposure (Wedemeyer and Yasutake, 1978). The need to draw on such data arises from the relative absence of data on ornamental fishes. During our experiment, koi carp larvae were exposed to an average NO_2 - N concentration of 0.211 mg/ L in NE for 3 months, which was higher than the 0.06 mg/ L limit reported for rainbow trout.

Unionized ammonia is also regarded as highly poisonous to fish (Arillo *et al.*, 1981). The permeability of the uncharged and lipid soluble unionized ammonia (NH_3) to plasma membranes is higher compared with the ionized form and therefore, is considered to be the more toxic form (Meade, 1985). Earlier studies have shown that common carp are relatively sensitive to unionized ammonia with a reported LC_{50} value of 0.44 - 1.9 mg/ L (Dabrowska and Sikora, 1986; Xu *et al.*, 1994). Although unionized ammonia was not measured in our experiment, it may be assumed that high temperature and pH levels during the entire growth period would block the ionizing process of NH_3 to the relatively non-toxic NH_4 - N (Ng *et al.*, 1992). In our study, the average NH_4 - N in NE was 0.753 mg/ L, when the average pH was 7.36 and the average temperature above 30^0 C. Under these conditions, percentage of NH_3 in water was estimated to be about 2% of the NH_4 - N (Emerson *et al.*, 1975), i.e., 0.015 mg/ L, which is below the threshold limit of 0.44 mg/ L. However, according to Parma de Croux and Loteste (2004), even an incidental increase in the pH to more than 8.0 in such situation could lead to high mortality due to significant increase in NH_3 toxicity. Mortality may also arise due to depressions of feeding when water quality is sub-standard (Asano *et al.*, 2003). Probably, these factors may have influenced the low survival rate (60.43%) of koi carp in the NE treatment.

Continuous supply of oxygen through aeration is known to promote nitrification in ponds, thereby lowering ammonia levels (Avnimelech *et al.*, 1986). Since most farmers in India cannot afford aeration facilities, water exchange is used as an alternative to maintain water quality. However, high level of water exchange could flush out nitrifying bacteria leading to reduced nitrification and increased ammonia concentrations (Diab *et al.*, 1992; Milstein *et al.*, 2001). Perhaps, the solution lies in low level of water exchange (only 5%, as experimented in the present study), but with increased frequency. From the present investigation, a daily water exchange rate of 100 L (WE1) appeared to be the most effective for koi carp tanks manured with poultry excreta. No water exchange (NE) result in water quality deterioration, deplete the plankton population and cause adverse impact on fish growth.

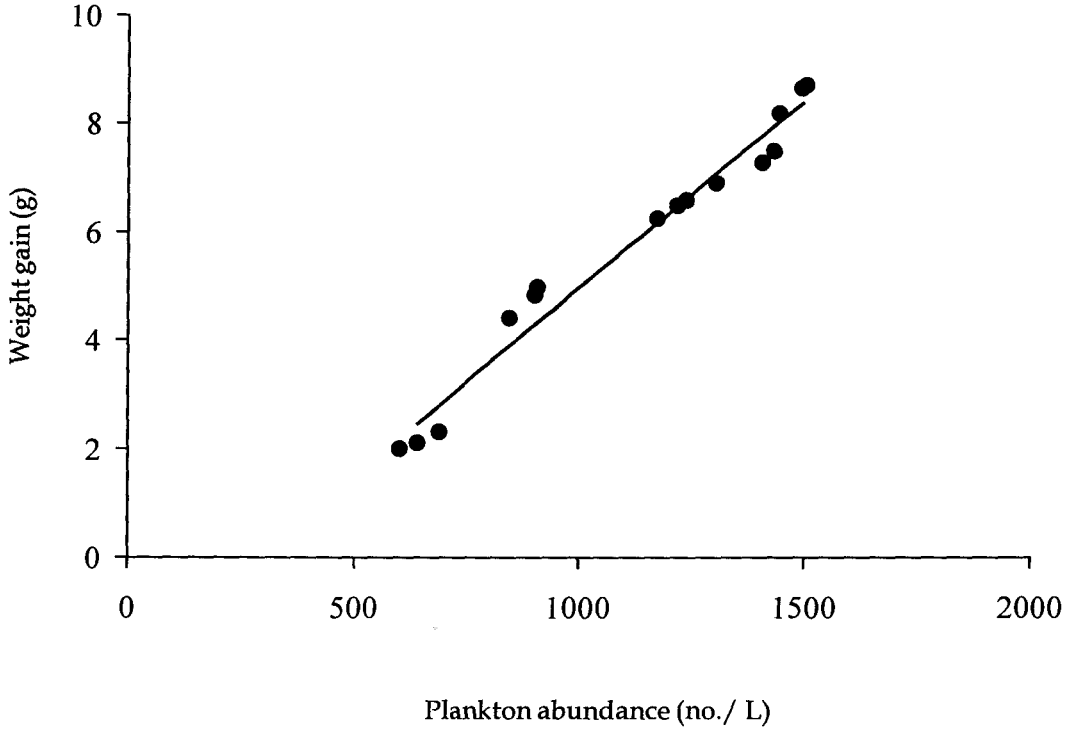

Figure 11 Relationship between weight gain of koi carp and plankton abundance in the five treatments.

5.3. Studies on the effect of stocking density on fish production (Experiment No. 3)

Growth rate of fish depends on many factors, but within the limits of the genetic growth potential of the species, they are principally a function of the availability of preferred food. Backiel and LeCren (1967), Hepher (1967), Bardach *et al.* (1972), Suresh and Lin (1992), Gress *et al.* (1996), Irwin *et al.* (1999), Metusalach *et al.* (1999), and Sharma and Chakrabarti (1999) indicated that growth of many species was density dependent and that there was an inverse relationship between stocking density and individual size of fish produced, primarily because the food supply had to be shared between individuals.

In our experiment, all the fish were fed to satiation. In addition, in the outdoor tanks, fish had access to algae, hence, food availability alone cannot have caused the differences in the growth rate between the different treatments. It is well known that carp fry respond quite rapidly to different stocking densities in their competition for space as well as food. Overstocking usually results in low survival rate, while reduced stocking leads to rapid growth of the fry (Rothbard, 1982; Rothbard and Yaron, 1995). Cage culture experiments in Israel have shown common carp to be extremely sensitive to stocking density (Feldlite and Milstein, 1999), where food availability was not a problem, but increased stocking density reduced the space volume available per fish. Violating behaviour requirements for space can affect growth through endocrine responses or disruption to feeding efficiency (Pankhurst and Van der Kraak, 1997; Schreck *et al.*, 1997). Fox and Flowers (1990) reported on increased losses due to cannibalism in juvenile walleye, *Stizostedion vitreum*, grown at high densities in intensive culture ponds. Cannibalism in many species of fish appears to be directly influenced by the availability of space and shelter (Smith and Reay, 1991; Herbert *et al.*, 2003).

Shelton *et al.* (1981) found that increasing stocking density had a profound negative impact on the growth of grass carp in small impoundments. Among ornamental fish, a similar effect of population density on growth rate was found by Olivier and Kaiser (1997) with juvenile swordtails fed to satiation, and Degani (1993) with angelfish, although, the later experiment was conducted indoors and the initial size of fish was more than 1 g. For our experiment, advanced larvae (0.14 ± 0.035 g) of koi carp were

selected, since, tropical fish breeders in India usually sell fish larvae (of 2 - 3 weeks age) to fish growers. Large-scale fish production involves fry stocking in earthen ponds from where marketable fish are harvested after 3 - 4 months of culture. One of the bottlenecks in this industry is the large-scale loss of fish during the post larval stage, where mortality rates are very high before the fish reach 1 g size.

Environmental conditions in the culture tanks were influenced by the stocking density. High accumulation of excrements and metabolic wastes from the fish led to significantly higher concentrations of nitrogen compounds and simultaneously lowered the DO in D4 and D5, compared to other treatments. Low dissolved oxygen is considered as one of the limiting factors to fish production. A clear-cut relationship between fish yields and increasing levels of aeration was documented for tilapia (Teichert-Coddington and Green, 1993). According to Stone *et al.* (2003), repeated exposure to low DO can slow the growth process in goldfish.

Different species are differently sensitive to nitrogen toxicants. One of the common problems in koi carp culture is depressions of feeding when water quality is substandard. Asano *et al.* (2003) observed periods of depressed feeding in koi carp when ammonia levels were high and dissolved oxygen was low. Although unionized ammonia was not estimated in our experiment, it is known to be in equilibrium with the development of ammonium ions in water (Barat and Jana, 1990). Nitrite is also toxic to many species of fish (Barat and Jana, 1991). Although daily water exchange helped in controlling ammonium and nitrite level in the tanks, the significantly higher growth and survival rates of koi carp in the D1, D2 and D3 treatments, compared to D4 and D5, could be influenced by the better water quality in terms of higher dissolved oxygen and lower levels of nitrogen toxicants. Jana and Barat (1992) reported marked changes in water quality due to high stocking load of fish in culture tanks. Physiological stress and impaired growth due to poor water quality associated with crowding have been reported in rainbow trout (Zoccarato *et al.*, 1994) and summer flounder, *Paralichthys dentatus* (King *et al.*, 2000).

The results from the probability distribution indicate that stocking koi carp at a density of 0.3 fish/ L, rather than at higher or lower densities, yielded the highest number of saleable fish. In contrast to food fish production, where producers focus primarily on

the total number of fish produced (Jolly and Clonts, 1993), ornamental fish can only be sold when they have reached a particular size. While the results consistently showed that increasing density reduced growth, it would appear logical to reduce the stocking density, so that a faster growth rate is allowed, and the fish can quickly reach the smallest marketable size (4 g).

However, no significant increase in price for koi carp that grow larger than the minimum marketable size (4 g) in India provide strong financial incentives for farmers to maintain fish at the smallest marketable size. The goal of production is to produce the highest number of fish of the given size (4 g) with consistently low size variation. Hence, although the weight gain of koi carp was considerably higher in D1 and D2 treatments, D3 (0.3 fish/ L) seemed to be the optimal density for stocking koi carp, since, the number of marketable fish was highest in that treatment.

The results suggest average size and survival rate of koi carp to be inversely related to stocking density, and, are in agreement with earlier studies with other fish species (Shelton *et al.*, 1981; Degani, 1993; Stone *et al.*, 2003). From the data obtained, a stocking density of 0.3 fish/ L appeared to be the most effective for stocking koi carp fry in tanks. The productivity should be measured in terms of number of marketable fish, which would favour a density of 0.3 fish/ L, compared to higher or lower stocking densities.

5.4. Studies on the effect of live-food treatment on fish production against conventional manuring regimen. (A) Comparative account of fish production throughout different seasons (culture periods) in a year, namely, winter, summer, monsoon and post monsoon (Experiment No. 4)

In agreement with earlier findings (Chapter 5.1 and 5.2), the linear relationship between the weight gain of koi carp and plankton abundance in the different treatments throughout the four growth trials exhibited a high correlation (r = 0.749; $P < 0.05$) (**Figure 12**).

Zooplankton is required as a first food for most cultured fish and contributes to faster larval growth and better survival (Prinsloo and Schoonbee, 1986; Lubzens *et al.*, 1987; Ludwig, 1999; Sharma and Chakrabarti, 1999; Al-Harbi and Siddique, 2001). In this experiment, exogenous introduction of live zooplankton significantly enhanced weight gain ($P < 0.05$) and reduced fish mortality ($P < 0.05$), compared to those of manured treatments (PM and CD) and control (C) in all seasonal trials. Although the amount of poultry manure used in the PM treatment was the same as applied in the plankton culture tanks from where plankton-rich water was transferred to the LF tanks, the zooplankton abundance (no./ L) was greater in LF, compared to PM in all seasons. In particular, the abundance of cladocerans was considerably higher (192.95% to 198.55%) in LF than the PM treatment throughout the four seasonal trials. Perhaps the selective grazing on young zooplankton, particularly cladocerans, by carp fry in the PM treatment reduced the chances of mass proliferation of zooplankton to the level as achieved in the plankton culture tanks in absence of any grazing pressure.

Another reason could be the water quality differences between the LF and the manured treatments. Earlier studies on life history parameters of *Daphnia* sp. and *Moina* sp., as also discussed in Chapters 5.1 and 5.2, suggest that growth, reproductive potentials and longevity of each species are affected by the nutrient conditions of the culture media. The maximum concentration of zooplankton in the LF treatment in all the seasonal growouts could be a consequence of improved water quality, expressed in terms of lower values of NH_4 - N and BOD, and higher values of DO and pH, which is conducive to fast reproduction of some of the major zooplankton constituting the main

food item of carps (Jana and Chakrabarti, 1993). In general, plankton intake by carp tends to rise with increasing food availability and ultimately attains a distinct pleatue level (Jana and Chakrabarti, 1988). Any uneaten plankton in the LF treatment would undergo prolific reproduction under such conducive environment. Higher weight gain of carp in PM, compared to CD in all the seasonal experiments can be explained by the significantly higher abundance of zooplankton ($P < 0.05$) in PM, which is directly related to the higher nitrogen input (also discussed in Chapter 5.1), and is in agreement with earlier findings (Kapur and Lal, 1986; Singh and Sharma, 1999).

Apart from food supply, temperature is one of the most important factors determining somatic growth of fish (Brown, 1957; Houde, 1997). Most of the fish growth models include growth as a function of temperature (Soderberg, 1992; Ney, 1993). If possible, fish often prefer habitats with both, temperatures, which maximize physiological growth processes (Wooton, 1992; Hofmann and Fischer, 2002; Hofmann and Fischer, 2003), and a food supply which is always available (Kramer *et al.*, 1997). In our experiment, average water temperature during the winter growout was 18.58^{0}C (range 16^{0}C - 25^{0}C; n=12). The range of optimal temperature for fish growth is species specific (Weatherley, 1990) and is considerably lower for stenothermic than eurythermic fish. Although the common carp (consumable varieties) can tolerate extreme and fluctuating temperatures, the optimal range has been suggested as 26.7^{0}C to 29.4^{0}C (McLarney, 1987).

Survival rates of carp ranged from 70.50% (C) to 95.50% (LF) in the winter trial, and were lower by 2% to 8% in the different treatments than that achieved in the summer trial. According to Kinne (1970) and Schmidt-Nielsen (1997), as temperature decreases to the lower thermal limit for an animal, mortality occurs as a result of failure of enzyme systems and respiration. Before the lethal temperature is reached, the physiological condition of the animal may be significantly affected at sublethal temperatures, resulting in its higher susceptibility to disease and stress. Therefore, mortality is likely to occur at temperatures higher than the lower thermal limit under culture conditions, as experienced in our study, where, during the winter trial, high mortality was recorded, even though the lower temperature limit for common carp has been reported to be as low as 0 - 0.7^{0}C (McLarney, 1987). The success of carp aquaculture depends not only

on survival, but also on growth rate of the cultured animals. In our experiment, weight gain of koi carp in all treatments was considerably lower in the winter trial compared to other seasons. In the LF treatment, under similar management conditions, the carp achieved 83.78% increase in weight gain during summer, compared to the winter growout. According to Horvath *et al.* (1992), metabolism and food demand of carp decreases gradually with decreasing water temperatures below 20°C, leading to lower growth rates.

Increased weight gain in other seasons, compared to winter has been reported with grass carp (Shrestha, 1999) and Indian Major Carps, rohu, mrigal and catla, *Catla catla* (Dhawan and Kaur, 2002 b). Usmani and Jafri (2002) obtained significantly lower digestibility rates for different food ingredients in two catfish species, *Clarias gariepinus* and *Heteropneustes fossilis* at 18°C than those obtained at 28°C or higher temperatures. According to El-Sayed *et al.* (1996), growth of nile tilapia was significantly reduced at temperatures below 21°C. Lower weight gain of koi carp during the winter trial in our experiment may also be related to the lower abundance (no./ L) of zooplankton in winter, compared to other seasons. Similar decrease in zooplankton abundance during winter has also been reported by Dhawan and Kaur (2002 b).

Significantly higher ($P < 0.05$) incidence of fish deformities (mostly scoliosis and bent fins) was obtained in the C treatment in all seasons. The percentage of deformed fish in the various treatments cannot be authentically explained with the available data. The absence of any earlier report with reference to deformities in koi carp related to its husbandry management makes it difficult to arrive at any conclusion. The deformities may have a genetic background since the experimental larvae were the offspring of a mixed commercial production from parents of different koi types that were randomly stocked in the tanks. It may also be environmentally induced. Significantly higher percent of fish with deformities in the control treatment could be attributed to lower abundance of plankton in the system throughout the year. Skeletal and other deformities are associated with nutritional deficiencies or imbalances in young fry and fingerlings fed on commercial diets and are rarely observed in ponds due to presence of live food (Malison, 2003). Another reason could be a possible leaching of nutrients out of the pelleted food applied in the control treatment. Goldblatt *et al.* (1979) demonstrated that

pelleted diets exhibit a remarkable loss of vital nutrients, such as vitamins and amino acids, within a short period of exposure to water. High incidence of deformities in fish fed with commercial diets has been reported for crucian carp (Myszkowski *et al.*, 2002).

From the results of the normal probability distribution, the LF treatment appeared to be the most productive treatment, followed by the PM and CD treatments. The control treatment, however, did not produce any marketable fish in the winter, summer and monsoon growout, and even in the post monsoon trial, only 4.52% of stocked fish in this treatment attained marketable size. In contrast to food fish production, where the total mass of fish produced determines productivity (Jolly and Clonts, 1993), ornamental fish can only be marketed once they have reached a particular size, provided they are free from any deformities and meet the aesthetic requirements of the industry. Low growth rate of koi carp in the winter trial ensured that none of the stocked fish in the manured or control treatments attained marketable size. From the present investigation, the winter season appeared to be the most unproductive, as only the LF system produced saleable fish. Throughout the year, the live-food treatment (LF) appeared to be the most effective for koi carp tanks, compared to tanks manured with poultry excreta (PM) or cow dung (CD), through maintenance of better water quality and greater abundance of zooplankton in the system.

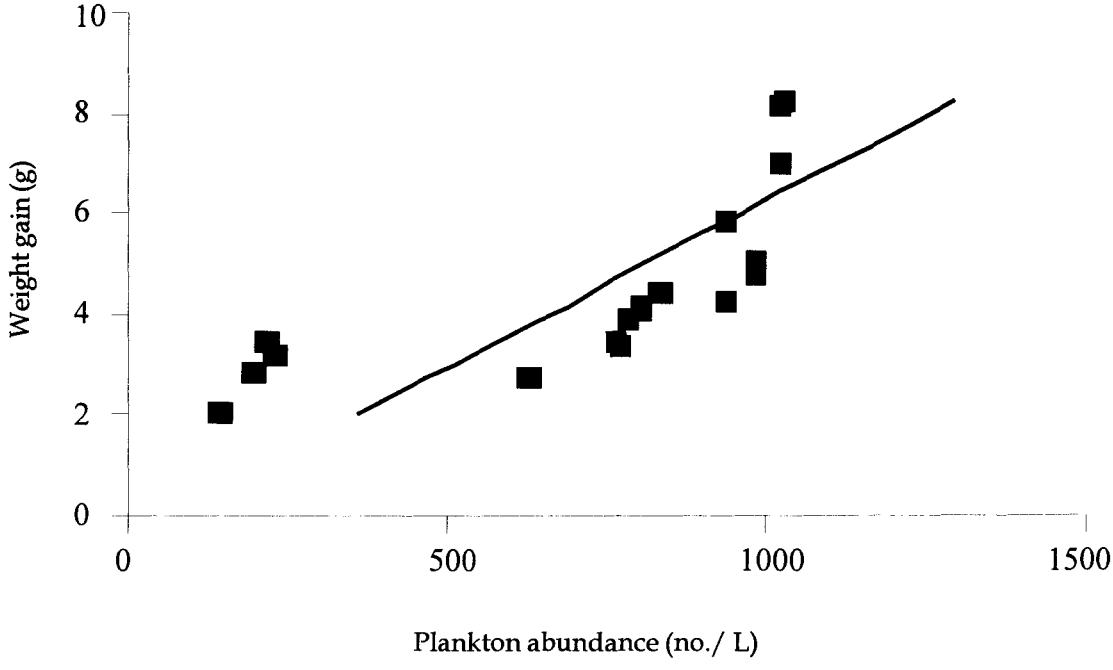

Figure 12 Relationship between weight gain of koi carp and plankton abundance in the four treatments during the different seasonal trials.

5.5. Studies on the effect of live-food treatment on fish production against conventional manuring regimen. (B) Comparative account of fish production in different culture systems, namely, concrete tanks and earthen ponds (Experiment No. 5)

This study shows that feeding of live zooplankton is potentially capable of supporting nearly 48.61% (ponds) to 66.92% (tanks) higher weight gain of carp than that obtained in the poultry manured treatments. Related to maximum plankton intake, the maximum concentration of plankton in the live-food units (in both tanks and ponds) was a consequence of improved water quality, expressed in terms of lower values of BOD and NH_4 - N, and higher values of DO and pH, which is conducive to fast reproduction of some of the major zooplankton constituting the food items of carp larvae (Jana and Chakrabarti, 1993), and also due to the regular introduction of live plankton.

All aquaculture production systems must provide a suitable environment to promote the growth of aquatic crop. Critical environmental parameters include the concentrations of DO, ammonia, nitrite and to a lesser extent nitrate, pH and alkalinity in the water of the production system (Losordo *et al.*, 1992). Although numerous literature are available on fish production in either pond or tank culture systems, very few authors have actually tried to compare the environmental parameters and fish production between both systems. Greater control over production has been the reason of increasing popularity of tank culture over the traditional method of pond fish production. However, in our experiment, manured ponds offered better environment for plankton reproduction in terms of lower NH_4 - N and NO_2 - N levels, compared to manured tanks. As also discussed in Chapters 5.1, 5.2 and 5.4, earlier studies on life history parameters of most cladocerans suggest that growth, reproductive potentials and longevity of many species are affected by the nutrient conditions of the culture media. High concentrations of NH_4 - N were found to restrict the occurrence of many small protozoans that are considered as excellent food for cladocerans (Pfister *et al.*, 2000). One of the possible reasons of higher NH_4 - N and NO_2 - N concentration in tanks could be that daily water exchange in tanks flushed out nitrifying bacteria, particularly *Nitrobacter* sp., leading to increased NH_4 - N concentrations and reduced nitrification (Avnimelech *et al.*, 1994). Significantly lower NO_3 - N concentrations in manured tanks, compared to manured ponds ($P < 0.05$) also lend support to this hypothesis.

In traditional pond culture, proper environmental conditions are maintained by balancing the inputs of fertilizers and feeds with the natural assimilative capacity of the pond. The pond's natural ecosystem, including photosynthetic algae and beneficial bacteria drives important biological processes that impact the daily oxygen cycle and provides a natural biofilter which breaks down harmful nitrogenous wastes (Losordo *et al.*, 1992). Although a 10 cm layer of soil was placed in the bottom of the experimental tanks, it failed to replicate the exact pond environment. Perhaps the stagnation of water in the tanks as compared to even limited wind driven mixture in the ponds caused the differences in the physico-chemical parameters of water in the two systems. A moderate manuring dose of 0.26 kg/ m^3 (corresponding to 2600 kg/ ha) every 10 days was applied to the culture tanks (TPM and TCD) and ponds (PPM and PCD) in this experiment. The traditional application rate of organic manures in Indian ponds in considerably high: 10000 kg/ ha as initial dose and a subsequent application of 5000 kg/ ha (Jhingran, 1991). According to Culver and Dabrowski (1998), optimal fertilization methods may vary from location to location. When the application exceeds the assimilatory capacity of the culture system, it leads to deterioration of water quality through accumulation of ammonia and nitrite (Hargreaves, 1998). In view of the similar manuring dose as applied to the tanks and ponds in the present experiment, it appears that the assimilatory capacity of the ponds was higher than that of tanks.

Under any management regime, higher weight gain and survival rates of koi carp were observed in the ponds, compared to culture tanks. Studies with other fish species have yielded similar results. Significantly lower growth rates of channel catfish, *Ictalurus punctatus* were obtained in concrete pools compared to earthen ponds (Shell, 1966). Although successful on a laboratory scale, tank culture of yellow perch (*Perca flavescens*) fingerlings is not widely practiced, and commercial production is carried out in ponds based on consumption of live food (Malison, 2003). Even in case of walleye, younger fry (upto 6.5 cm) are usually cultured in ponds as the growth rate is better, compared to tanks (Summerfelt, 1996). However, it may be difficult to maintain a steady growth rate of older fry (size > 10 cm) in ponds without the addition of extra feed (Jorgensen, 1996). According to Kinnunen (1996), pond culture is economically effective for the production of young walleye fingerlings, even if it may not be the choice for older size fingerlings (Raisanen, 1996). In case of ornamental carp culture, fish are

marketed after attaining the minimum marketable size, i.e. 4 g (about 6 - 7 cm), and as evident from our experimental results, pond culture seems to be the better alternative, compared to tanks for any particular management regime.

Significantly higher percentage of deformed koi carp in the control treatments could be attributed to the commercial diet applied in these units. This is in agreement with earlier findings (Chapter 5.4). According to Malison (2003), skeletal and other deformities are associated with nutritional deficiencies or imbalances in tank cultured fry and are rarely observed in ponds due to presence of live food. In our experiment, significantly higher ($P < 0.05$) percentage of deformed carp was observed under any management regime in tanks, compared to ponds.

Both management regime and the type of culture system had an effect on the number of marketable fish. The live-food treatments for both tanks and ponds appeared to be highly productive, followed by the PPM treatment. Most treatments maintained in tanks were unproductive because of the significantly lower weight gain of koi carp. One of the possible reasons for the slow growth could be the culture season i.e. winter (December - February), and it appeared that a prolonged culture period could increase the productivity of most of the culture systems in terms of marketable fish produced.

Talking all the aforementioned aspects into account, it can be recommended to introduce live zooplankton into culture units for raising koi carp larvae. Due to differences in their basic nature, as also the differences in the size of the culture tanks and ponds employed, it was not possible to derive conclusions about which system was more productive. However, from the experimental results, earthen ponds appeared to be better alternative to concrete tanks for manure application through maintenance of better water quality due to their higher assimilatory capacity and greater abundance of plankton.

5.6. Studies on the effect of live-food treatment on fish production against conventional manuring regimen. (C) Examination of food selection and food preference of cultured fish in the different treatments (Experiment No. 6)

Electivity indices ranging from - 0.3 to + 0.3 are generally considered not significantly different from zero, and thus indicate non-selectivity feeding (Lazzaro, 1987). According to this interpretation, the koi carp larvae in our experiment did not show any significant food selectivity towards most planktonic organisms. Analyzed by plankton types, a strong rejection (below - 0.3) was observed only towards phytoplankton in LF and PM, and towards rotifers in LF and CD. There was no incidence of strong selection (above + 0.3). Analyzed by individual plankton, there was only one incidence of strong positive selection (towards *Daphnia* in CD).

However, other authors (Xie and Takamura, 1996; Serajuddin, 2000) have defined electivity values above + 0.01 as positive selection and below - 0.01 as negative, thus reducing the non-selectivity feeding range to - 0.01 to + 0.01. According to this definition, food selectivity of koi carp larvae was clearly demonstrated in the experimental results: positive selection for cladocerans and negative selection for the other groups, although the extent of selection or rejection differed markedly from one treatment to another.

Since cladocerans were found in larger proportions in the diet than in the environment in all the treatments, it implied that cladocerans constituted an important source of natural food for koi carp larvae held in any culture system. Because of the positive selection of cladocerans in all the treatments, it can be suggested that koi carp larvae had a preference for cladocerans as food despite copepods being the dominant plankton group in the environment of all treatments, except LF. This shows that koi carp larvae do not necessarily feed on the most abundant type of plankton.

Feeding strategy of planktivores is based on the structure and functioning of their branchial feeding apparatus viz. gill rakers (Serajuddin, 2000). The presence of mucous, which helps in consolidation and transportation of food items, could also improve the retention efficiency of the filter. Besides the characteristics such as shape and size of suspended particles and alteration capabilities of mesh size of gill rakers also play an important role in food retention (Serajuddin, 2000). Rejection of food items might be due to their size, which may be beyond the fish's capacity to deal with. Considering

that koi carp larvae in our experiment were relatively young (0.13 - 8.67 g), the size of their mouths could be a constraint while consuming larger sized planktonic organisms. It is known that in nature, small mouth size of carp fry (Dabrowski and Bardega, 1984) acts as a constraint for optimal diet breadth during early stages (Werner, 1974).

The avoidance of food organisms may also be linked to distastefulness of food, especially when fish probes the aggregation of food items, as demonstrated by the differential secretion of mucous by grass carp under varied food conditions (Omarova and Lazareva, 1974). Negative selectivity (PM, CD and C) to outright rejection (LF) of phytoplankton by koi carp larvae is in agreement with earlier experiments with other fish species, namely, catla (Jafri and Mustafa, 1975), brown trout, *Salmo trutta* (Fitzmaurice, 1979) and common carp (Chakrabarti and Jana, 1990), where negative selection for phytoplankton were reported. The need to draw on such date arises from the relative absence of data on ornamental carps.

Higher weight gain, SGR and survival rate of koi carp in the LF treatment ($P < 0.05$) could be attributed to the significantly higher abundance of cladocerans in that treatment. The maximum concentration of zooplankton in the LF treatment was a consequence of improved water quality, expressed in terms of lower values of BOD, ammonium and nitrite, and higher values of DO and pH, which is conducive to fast reproduction of some of the major zooplankton constituting the main food item of carps (Jana and Chakrabarti, 1993), and also due to the regular introduction of live plankton. Plankton intake of planktivorous fishes varies with different feeding conditions. Jana and Chakrabarti (1990) reported the plankton intake of common carp in the live-food system was higher than in manured or control system.

A direct relationship between plankton intake and the average body weight of carp has been demonstrated by Chakrabarti and Jana (1991). Similar results were also obtained in our earlier experiments (Chapter 5.1, 5.2 and 5.4). The significantly lower weight gain, SGR and survival rates in the C treatment may be due to insufficient quantity of plankton in the system. Although an imported pelleted feed was applied in this treatment, it seems from the experimental results that the larvae did not prefer the pelleted feed. Similar results were obtained in earlier experiments (Chapter 5.4 and 5.5).

The deformities observed were mostly scoliosis and bent fins. As also discussed in Chapters 5.4 and 5.5, the significantly higher percent of fish with deformities in the control treatment could be attributed to lower abundance of plankton in the system. Even the number of marketable fish was highest in the LF treatment and is in agreement with the findings in Chapter 5.4 and 5.5.

From the findings of the present investigation, raising koi carp larvae in live-food ponds (LF) with introduction of exogenous plankton appears to be a better alternative than the conventional system of direct application of poultry manure (PM) or cow dung (CD) in the ponds.

5.7. Studies on the effect of live-food treatment on fish production against conventional manuring regimen. (D) Estimation of bacteriological counts of water and bottom sediment in the different treatments (Experiment No. 7)

The microbiological status of the water in which fish culture takes place depends on a wide variety of factors influencing the environment, the most important being the organic matter content (Rheinheimer, 1980; Sugita *et al.*, 1985 b; Zmyslowska *et al.*, 2003). Variations of heterotrophic bacteria in the water samples of the four treatments were the results of differences in management practices causing differences of organic loadings in the pond system. Thus the management regimes receiving organic manures (PM and CD) recorded significantly higher populations of total heterotrophic bacteria, compared to other treatments. The highly productive nature of the manured ponds was also supported by the greater abundance (no./ L) of total plankton, compared to the control treatment.

Low counts of heterotrophic bacteria in ponds not receiving any organic manuring have been reported earlier by many authors (Barat and Jana, 1990; Jana and De, 1990; Barik *et al.*, 2001; Majumdar *et al.*, 2002). As such, the control system appeared to be less productive, as also indicated from the significantly lower plankton abundance ($P < 0.05$), compared to the manured treatments. According to Ludwig (1999), when organic fertilizers are added to a pond, they are decomposed by bacteria and the water rapidly gains nutrients from the bottom. The released nutrients are rapidly utilized by phytoplankton and other bacteria, which are simultaneously grazed by single cell protozoan and other zooplankton. In control ponds, as also observed in our study, there are few nutrients, and hence few living organisms.

Although heterotrophic bacteria and phytoplankton are important components in the cycling of organic matter and inorganic nutrients in aquatic ecosystems, they may affect each other positively or negatively, depending on the nutrient conditions of their environment (Wang and Priscu, 1994; Kamjunke *et al.*, 1997; Duvall *et al.*, 2001). Because bacteria have a high surface area to volume (Currie and Kalff, 1984), it has been suggested that bacteria should be superior competitors with phytoplankton for nitrogen and phosphorus (Elser *et al.*, 1995). However, in our experiment, higher abundance of total heterotrophic bacteria in the manured treatments was correlated with high phytoplankton abundance (in PM, $r = 0.625$, $P < 0.01$; in CD, $r = 0.588$, $P < 0.01$).

Brett *et al.* (1999) suggested that the underlying mechanisms behind the positive correlation between phytoplankton and bacteria are tangled in complex interactions between factors such as inorganic nutrient concentrations, organic nutrient availability, protozoan bactivory, availability of physical substrate, as well as light and temperature. Such complications could prevent augmented bacterial populations from having significant effects on phytoplankton. In experiments by Cottingham *et al.* (1997), bacteria did not buffer phytoplankton responses to nutrient enrichment. In view of the continuous grazing pressure on bacteria and phytoplankton by zooplankton and on zooplankton by fish larvae, it is very difficult to estimate the exact population density of bacteria, phytoplankton or zooplankton in any aquatic system. However, the overall results clearly demonstrate the importance of pond management on the growth responses of heterotrophic bacteria.

The abundance of heterotrophic bacteria in the pond sediments did not differ significantly from one system to another. It implies that the sediment of all fish ponds in our experiment, regardless of the farming system, contained the optimal amount of essential nutrients necessary for massive growth of heterotrophic bacteria. Jana and De (1990) obtained similar results in the sediment of traditional and manure-treated ponds. According to Jinyi *et al.* (1988), because of the sedimentation of applied manure and pond mud in both manure-applied and control ponds, the amount of bacteria in the water column decreases from pond bottom to the surface layer of water with the continuous release of microorganism from the sediments. Similar results were also observed in our study.

Greater abundance of *Aeromonas* sp. and *Pseudomonas* sp. in the water and sediments of PM and CD, compared to the control treatment, indicate their sewage character. Very high counts of *Aeromonas* sp. and *Pseudomonas* sp. in ponds manured with animal excreta have been reported by many authors (Cloete *et al.*, 1984; Jinyi *et al.*, 1987; Jinyi *et al.*, 1988; Hamza *et al.*, 1998). The introduction of live plankton in the LF treatment, however, significantly reduced the population of total heterotrophic bacteria, as well as *Aeromonas* and *Pseudomonas* in both water and sediment, compared to the manured treatments.

The water quality was also influenced by management conditions. Significantly high NH_4 - N in the PM and CD treatments could be related to the greater abundance of heterotrophic bacteria in these treatments, since apart from ammonifying bacteria, which was not enumerated in our experiment, many heterotrophic bacteria are known to utilize nitrogen-rich substrate and release ammonia or ammonium salts (Jana and Barat, 1983). Yao and Zhaoyang (1997) reported that the contact layer between pond mud surface and water is the major source of nutrition. The organic nitrogen decomposed to NH_4 - N by bacterial activity adheres to the surface of the mud before being released in the water, where it continuously rises to the surface of the water and escapes to the air (Blackburn and Henriksen, 1983; Mei *et al.*, 1995).

Depletion of dissolved oxygen after manure application often leads to heterotrophic organisms in the water utilizing NO_3 - N as electron receptor instead of oxygen, thus converting it to nitrite (Boyd, 1990). Higher concentration of BOD, NH_4 - N, NO_2 - N and other nutrients, along with the higher counts of *Aeromonas* sp. and *Pseudomonas* sp. in the manure treated ponds may have lowered the grazing activity by the carp, compared to the LF treatment. Neutral to acidic pH in the water of majority of the treatments could be related to the acidic nature of water bodies in North Bengal (Nath *et al.*, 1994; Jha and Barat, 2003; Jha *et al.*, 2003).

Higher weight gain, SGR and survival rate of koi carp in the LF treatment could be attributed to the better water quality and significantly greater abundance ($P < 0.05$) of zooplankton in that treatment. The deformities observed were mostly scoliosis and bent fins. Significantly higher percentage of deformed koi carp in the control treatment could be attributed to: (1) low counts of live plankton, and (2) the commercial diet applied in that treatment, and is in agreement with earlier observations (Chapter 5.4, 5.5 and 5.6). Management condition also had an effect on the number of marketable fish and the LF treatment appeared to be more productive, compared to the manured treatments or control.

All aquaculture production systems must provide a suitable environment to promote the growth of aquatic crop. Although application of organic manure does not directly cause bacterial diseases in fish, the significantly greater abundance of pathogenic bacteria (*Aeromonas* sp. and *Pseudomonas* sp.) in the water and sediments of the manured

treatments (PM and CD) imply, should the fish resistance to disease be low, the possibility of occurrence of bacterial disease is higher in these treatments. Therefore, proper pond management should be observed to prevent any chance of bacterial disease.

Though it is well known that high fish yield in culture systems can be achieved by higher abundance of plankton, perhaps it may not be possible to fertilize with manure because this may reduce water quality. Intensive stocking in ornamental fish ponds in India requires a standard water quality to be maintained throughout, so that fish growth is not adversely affected. In view of the financial constraints of marginal farmers who cannot afford modern aeration or waste-treatment equipments, raising of ornamental carp larvae in ponds fed exogenously with zooplankton is of considerable significance because such feeding would support high rates of survival and production through maintenance of better water quality and greater abundance of zooplankton in the system.

5.8. Studies on experimental ornamental fish polyculture. (A) Behavioural responses of two popular ornamental carps, koi carp and goldfish to monoculture and polyculture conditions in aquaria (Experiment No. 8)

The depths occupied by the two fish species under different experimental conditions were a function of: (1) Species-specific differences in mean depth preference. This could explain for the similarities in depth preference of monocultured fish of any particular species within two experimental batches in the absence of food. (2) Species-specific changes in depth in response to the addition of food. The preferences of the bottom tank levels by both fish species in the first batch in the presence of tubifex worm lend a support to this hypothesis. The reduction in mean depth recorded for monocultured koi carp in the second batch in the presence of plankton may also be influenced by the upward movement of the species towards the surface for grazing on the maximum amount of zooplankton available at the position of the tank where it was administered, before the live plankton could disperse to other areas of the tank. (3) Species-specific changes in depth in response to polyculture conditions. This was probably the most important factor and is associated with aggressive behaviour of individual species.

The two species exhibited considerable variation in the extent and type of aggression displayed. Goldfish in monoculture treatments appeared less aggressive, compared to monocultured koi carp in both experimental batches. Even in the polyculture treatments, goldfish attacked conspecifics or other species (koi carp) very rarely. On the other hand, koi carp were overwhelmingly more aggressive. The frequency of attack increased significantly in the presence of food. Food has shown to increase the rates of aggression in blue gourami (Syarifuddin and Kramer, 1996), gambusia, *Gambusia holbrooki* and swordtail (Warburton and Madden, 2003). The broader diversity of species-specific behaviours and salient stimuli may also have encountered heightened levels of activity. In a study of conspecific and interspecific interactions between brook trout, *Salmo gairdneri* (*Oncorhynchus mykiss*), Newman (1956) postulated that the presence of food increased feeding activity, which in turn increased aggressive activity as the focus of attacks was displaced from the food to fellow fish of both species. He further noted that feeding fish

displayed some movements that are associated with aggression, such as body undulations, swift darting and biting, and suggested that such movements constituted sign stimuli eliciting attacks from other species.

The significantly higher rate of attacks in the polyculture treatments compared to monoculture conditions for both batches of fish question the very logic behind stocking koi carp and goldfish together. Although the impact of nipping on spinal and caudal abnormalities, or fin damages were not estimated in the present experiment, it could be suggested that sustained attacks, particularly on goldfish by koi carp under polyculture conditions could lead to stress and increased rate of deformities in pond polyculture. As discussed earlier, ornamental fish need to be visually attractive to be acceptable in the market, and deformed or stressed fish could be aesthetically unattractive to potential customers.

The two food items used in the two experimental batches (tubifex and plankton) were selected with a due consideration to the food availability under pond conditions. The impact of aggressive behaviour of koi carp was clearly demonstrated by the increased level of attack on goldfish in the polyculture treatments in both the experimental batches. Working with introduced poeciliid (gambusia) and native Australian fish, *Pseudomugil signifer*, Howe *et al.* (1997) observed that the prerequisites for competition exist when mixed populations of fish species are trapped in shrinking ponds during drought. In India, ornamental fish ponds are generally much smaller, compared to other aquaculture ponds, and competition pressure may severely affect the production status of the 'non aggressive' species under such confined habitat conditions.

Although the present laboratory-based findings should not be applied in a precise predictive way to judge interspecific interrelationships in ponds, they do illustrate behavioural mechanisms by which koi carp may negatively impact goldfish under confined conditions in ponds.

5.9. Studies on experimental ornamental fish polyculture. (B) Comparison of food selection and growth performance of koi carp and goldfish in monoculture and polyculture rearing in tropical ponds (Experiment No. 9)

The similarity in the types of organisms present in the gut of koi carp and goldfish may be due to the fact that all the ponds were maintained under similar management conditions and the food (plankton-rich water) was supplied form a series of ponds, also similar in size and management. Hence there was no difference in food resource between the experimental ponds. Water quality also was quite similar in all the treatments. Lower pH and dissolved oxygen in 100%K and 90%K-10%G treatments may be explained by koi carp stirring up mud from the bottom. Although earlier reports on koi carp are lacking, other carp species are known to create management problems in fish ponds by stirring up pond bottoms, thereby releasing nutrients from the soil (Wahab *et al.*, 2002), creating turbidity and lowering dissolved oxygen (Lutz, 2003).

In the present study, goldfish showed better weight gain in monoculture, compared to polyculture treatments, unlike the koi carp, which recorded no significant differences in growth parameters between different treatments. Within the different polyculture treatments, the highest growth for goldfish was recorded in 10%K-90%G. That polyculture affected goldfish production is clear from the experimental results, however, absence of any earlier report relating to polyculture of ornamental carps in tropical pond conditions makes it difficult to draw conclusions about factors responsible for this reduced growth rate and production. One of the possible reasons could be differences in the food selection by the two species, and competition for food between them under polyculture conditions.

The koi carp diet in the monoculture treatment (100%K) consisted of twelve genera of plankton, which was reduced to fewer species in the different polyculture treatments. A rejection of phytoplankton was observed in all treatments, and is consistent with earlier findings (Chapter 5.6). However, four genera of phytoplankton were identified in koi carp intestines collected from 100%K, compared to two genera in 90%K-10%G and 70%K-30%G, and one in each of the 50%K-50%G, 30%K-70%G and 10%K-90%G treatments. Greater diversity of food in the guts of monotypic populations indicate that

segregation by cohabiting fish species results in consumption of a narrower range of food items in polytypic communities, than when only one species is present (Andrusak and Northcote, 1971; Clady, 1981). Being the more aggressive species in polyculture, koi carp could select its preferred group of plankton (cladocera) within this narrow range, and the electivity towards cladocerans recorded almost similar in all the treatments.

In goldfish, phytoplankton was absent and only six genera of zooplankton were obtained in the intestines of monotypic populations (100%G). The plankton diversity significantly increased ($P < 0.05$) in the gut content of polytypic goldfish communities. However, greater diversity of the diet does not necessarily suggest better feeding conditions, since fish populations often consume a greater variety of food items under adverse conditions than when food supplies are unlimited (Ivlev, 1961). Greater diversity of plankton from the guts of polycultured goldfish may be influenced by the consumption of their preferred food (cladocera) by the koi carp. In monoculture, goldfish recorded a very strong selection for the cladoceran, *Daphnia* (0.346) and the genera contributed 35.10% of the gut contents in 100%G, which was significantly reduced in polycultured goldfish ($P < 0.05$), recording only 19.04% in goldfish gut content in 90%K-10%G, with an electivity of 0.055. Simultaneously, there was increased consumption of copepods, rotifers and phytoplankton in polyculture, compared to monoculture.

Behavioural observations in the earlier experiment (Experiment No. 8) showed that koi carp were more active in the polyculture treatments, compared to monoculture. Similar aggressive behaviour of polycultured koi carp was also noted during the present study and could account for the increased rate of deformities and fin damages observed in goldfish in the polyculture treatments. However, it was not possible to document details of the interspecific interactions between koi carp and goldfish in pond conditions. The deformities in both species were mostly scoliosis, spinal and caudal abnormalities and bent fins, and could not have been induced environmentally, since the management conditions of all the treatments were similar. As discussed earlier, ornamental fish must be visually attractive to be marketable and deformed or damaged fish are not saleable, even if they attain marketable size.

The main tool for managing polyculture systems and maximizing fish production is the knowledge of fish-fish and fish-environment quantitative relationships (Milstein, 1992). The selection of fish species is therefore very important. One problem frequently encountered in polyculture involves the overlap of food or habitat preferences among species or even downright antagonism (Lutz, 2003). Brummett and Alon (1994) indicated that although growth of redclaw crayfish *Cherax quadricarinatus* was not adversely affected by the presence of nile tilapia in polyculture, tilapia growth and food conversion were significantly impacted by redclaw crayfish. Yashouv (1968) related similar problems encountered in polyculture of tench, *Tinca tinca* and common carp. In spite of feed being applied to ponds, the two species apparently competed for the same resources and carp production was reduced when tench was present. Studies on stable carbon and nitrogen isotope values from ponds polyculturing silver carp, *Hypophthalmichthys molitrix* and bighead carp, *Aristichthys nobilis* in China suggested that there were various degrees of dietary overlap between two species with an average of 60% of their food from the same trophic level (Gu *et al.*, 1996). In another experiment, Mattson (1998) recorded similar feeding preferences in *Oreochromis shiranus* and *Barbus paludinosus*, when offered an array of planktonic food in aquaria. In a study on polyculture of tench, common carp and bigmouth buffalo, *Ictiobus cyprinellus*, Adamek *et al.* (2003) observed food competition between tench and carp (60.8%) and between tench and bigmouth buffalo (47.4%). Vromant *et al.* (2002) recorded significant interspecific competition between nile tilapia and common carp in polyculture systems in intensively cultivated rice fields. The need to draw on such data arises from the relative absence of literature on ornamental carp polyculture. Whatever preliminary data available suggest that both koi carp and goldfish are likely to prefer the bottom tank level (Sandford, 1998) and eat similar food items (Axelrod and Vorderwinkler, 1970).

Another problem with polyculture wherever labour costs are relatively high involves handling and sorting species at harvest. In our experiment, every pond was netted three times at harvest, which could have aggravated fin damages. According to Milstein (1992), polyculture is the appropriate technique when the goal is production of low-cost fish. When the goal is production of more expensive fish, monoculture simplifies the management (Wohlfarth and Schroeder, 1979; Hepher and Pruginin, 1981). The financial risks associated with each species in the different combinations require proper evaluation (Milstein, 1992).

Polyculture had a direct effect on the number of marketable fish. The results clearly indicate that monoculture treatments yielded the largest number of saleable fish. As also described earlier, ornamental fish can only be sold once they have reached a particular size (4 g or more). From the present study, a diminishing return becomes apparent in ornamental carp polyculture, compared to monoculture. Besides, keeping in view of the dietary similarities of koi carp and goldfish, and the aggressive nature of koi carp in polycuture, possibly leading to increased deformities and lower weight gain and SGR of polytypic goldfish communities, it is suggested to refrain from polyculture of goldfish and koi carp until further documentations relating to stocking and standard management of polyculture of ornamental carps are available.

The present *thesis* entitled, "Effect of different management regimes on the survival and growth of exotic ornamental fish, koi carp (*Cyprinus carpio* L.), under tropical conditions" embodies a study of standardization of culture technology for exotic ornamental fish, koi carp (*Cyprinus carpio* L.), under tropical conditions in India. This thesis consists of 175 pages. The main findings of the *thesis* are summarized as follows:

- ✓ An application rate of 0.26 kg/ m^3 every 10 days, appeared to be the most suitable for manuring koi carp tanks with either cow dung or poultry excreta. Higher application rates reduced water quality, depleted the plankton population and caused adverse impact on fish growth.
- ✓ A water exchange regime of 100 L (5% of water volume) every day appeared to be the most effective for koi carp tanks (capacity: 2000 L) manured with poultry excreta. No water exchange (NE) resulted in water quality deterioration, depleted the plankton population and caused adverse impact on fish growth.
- ✓ A stocking density of 0.3 fish/ L appeared to be the most effective for stocking koi carp fry in tanks. The productivity was measured in terms of number of marketable fish, which favoured a density of 0.3 fish/ L, compared to higher or lower stocking densities.
- ✓ Raising koi carp larvae in culture tanks or ponds with a regular supply of exogenous zooplankton appeared to be a better alternative compared with the conventional system of direct application of poultry manure or cow dung, through maintenance of better water quality and greater abundance of zooplankton in the system.
- ✓ Winter (temperature range 16^{0}C - 25^{0}C; average 18.58^{0}C) appeared to be the most unproductive season for culturing koi carp in Jalpaiguri district of West Bengal, India.
- ✓ Due to differences in their basic nature, as also the differences in the size of the culture tanks and ponds employed, it was not possible to derive conclusions about which system was more productive. However, from the experimental results, earthen ponds appeared to be better alternative to concrete tanks for manure application through maintenance of better water quality due to their higher assimilatory capacity and greater abundance of plankton.

- ✓ As evident from the electivity index, koi carp larvae showed a positive selection for cladocerans while electivity towards copepods was generally negative although the copepods were more abundant in the environment of the manured ponds, compared to cladocerans. Rotifers and phytoplankton was also avoided.

- ✓ Average counts of heterotrophic bacteria in the water of poultry excreta or cow dung-manured ponds were significantly higher than the live-food or control treatments. The development of *Aeromonas* sp. and *Pseudomonas* sp. were also higher in the manured treatments. Significantly lower abundance of *Aeromonas* sp. and *Pseudomonas* sp. in the live-food treatment considerably lowered any possibility of occurrence of bacterial disease.

- ✓ Koi carp and goldfish tended to swim at different depths in absence of food under monoculture conditions. However, species-specific changes in depth were observed in response to addition of food and polyculture conditions. The two species exhibited considerable variation in the extent and type of aggression displayed, with koi carp being the more aggressive species and the impact of aggressive behaviour of koi carp was conspicuous by the increased level of attack on goldfish in different polyculture trials.

- ✓ Goldfish showed significantly better weight gain and survival in monoculture compared to polyculture, while the koi carp recorded no significant differences in the growth parameters between the monoculture and polyculture treatments. Both koi carp and goldfish exhibited similarities in food preference under monoculture conditions. However, in polyculture treatments, the food selection of goldfish significantly altered. Keeping in view of the dietary similarities of koi carp and goldfish, and the aggressive nature of koi carp in polyculture, it is suggested to refrain from polyculture of goldfish and koi carp until further documentations relating to stocking and management of polyculture of ornamental carps are available.

Overall, the information presented in this *thesis* should help farmers to overcome some of the difficulties encountered with the commercial production of koi carp in India.

Annexure I

Composition of culture media

1. Total Aerobic Heterotrophic Bacteria

Viable heterotrophic bacterial populations were grown in standard nutrient agar media having the following composition (APHA, 1998):

Peptone	10.0 g
Beef extract	1.5 g
Sodium chloride	2.0 g
Agar (Himedia Laboratories, RM 666)	20.0 g
Distilled water	1000 mL
Final pH (at 25^0 C)	7.2 ± 0.2

The medium was sterilized in the autoclave at 15 lbs pressure (121^0 C) for 15 minutes.

2. *Aeromonas* Bacteria

Aeromonas Isolation Medium Base (Himedia Laboratories, Mumbai; M 884) was used for selective differential isolation of *Aeromonas* sp. from water and sediment samples. The medium composition is as follows:

Ingredient	g/ L
Tryptone	5.00
Yeast extract	3.00
L-Lysine hydrochloride	3.50
L-Arginine hydrochloride	2.00
Inositol	2.50
Lactose	1.50
Sorbose	3.00
Xylose	3.75
Bile salts	3.00
Sodium thyosulphate	10.67
Sodium chloride	5.00
Ferric ammonium citrate	0.80
Bromo thymol blue	0.04
Thymol blue	0.04
Agar	12.50
Final pH (at 25^0 C)	8.0 ± 0.2

28.15 g of the medium was suspended in 500 mL of distilled water and heated to dissolve the medium completely. It was then cooled to 50^0 C and one vial of Aeromonas Selective Supplement (Himedia Laboratories, Mumbai; FD 039) was aseptically added and mixed.

3. *Pseudomonas* Bacteria

Pseudomonas Isolation Agar (Himedia Laboratories, Mumbai; M 406) was used for selective and primary isolation of *Pseudomonas* sp. from water and sediment samples. The medium composition is as follows:

Ingredient	**g/ L**
Peptic digest of animal tissue	20.00
Magnesium chloride	1.40
Potassium sulphate	10.00
Triclosan (Irgasan)	0.025
Agar	13.60
Final pH (at 25^0 C)	7.0 ± 0.2

45.03 g of the medium was suspended in 1000 mL of distilled water containing 20 mL Glycerol and boiled to dissolve. Then it was sterilized by autoclaving at 15 lbs pressure (121^0 C) for 15 minutes.

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Effect of Different Application Rates of Cowdung and Poultry Excreta on Water Quality and Growth of Ornamental Carp, *Cyprinus carpio* vr. *koi*, in Concrete Tanks

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Abstract

Larvae (0.09±0.025 g) of Koi carp (*Cyprinus carpio* vr. *koi*) were cultured in outdoor concrete tanks for 90 days. Individual weight gain and survival rates were compared among a control (C), three treatments manured every 10 days with cowdung, applied at 0.13 kg/m^3 (C1), 0.26 kg/m^3 (C2), and 0.39 kg/m^3 (C3), and three treatments manured every 10 days with poultry excreta, applied at 0.13 kg/m^3 (P1), 0.26 kg/m^3 (P2), and 0.39 kg/m^3 (P3). Weight gain of Koi carp stocked at P2 was significantly higher than other treatments. There was a significant difference in survival of Koi carp among the treatments, ranging from 65.5% to 86% in C and P2, respectively. The C3 and P3 treatments yielded significantly higher values of specific conductivity, $NH_4 - N$, $NO_2 - N$, and $PO_4 - P$, and significantly lower values of dissolved oxygen than the other treatments. Zoobenthos population was low in all the treatments. The results suggest that application rate of 0.26 kg/m^3 every 10 days seems to be the most suitable for Koi carp tanks manured with both cowdung (C2) and poultry excreta (P2), through maintenance of better water quality and greater abundance of plankton in the system. Suitable environment in C2 and P2 resulted in significantly better growth of Koi carp than other treatments.

Key words: Koi carp, *Cyprinus carpio* vr. *koi*, cowdung, poultry excreta, application rate, plankton abundance, zoobenthos

Introduction

In recent decades, the market for ornamental fish has grown steadily. The annual global trade value has been estimated to amount to US $ 9 billion (Swain and Jena, 2002). The culture of Koi carp (*Cyprinus carpio* vr. *koi*) is rapidly growing in India. The term 'Koi' refers to many strains of ornamental carp that have been genetically selected over many generations (Feldlite and Milstein, 1999).

A common approach for increasing fish production in ponds is the direct application of fertilizer, which enhances production of plankton, a natural food item for fish (Chakrabarti and Jana, 1998). Pond fertilization practices using animal wastes are widely used in many countries to sustain productivity at low cost (Gupta and Noble, 2001; Majumder *et al.*, 2002). Among manure used, chicken's is preferred because of its ready solubility and high level of phosphorus concentrations (Knud-Hansen *et al.*, 1991). Soluble organic matter supplied to ponds by using manure stimulates phytoplankton growth (Sevilleja *et al.*, 2001). Moreover it increases biomass of zooplankton and benthic organisms (Atay and Demir, 1998). In India, however, cowdung is the most common organic manure applied to fish ponds (Singh and Sharma, 1999).

Cyprinid larvae are known to prefer natural food items such as free living protozoa and rotifers, and larger planktonic organisms like cladocerans and copepods at fry and fingerling stage (Jhingran and Pullin, 1985). Several pond management techniques have been developed to improve environmental conditions for cyprinid fry (Rothbard and Yaron, 1995). Among the techniques, manure usage at different rates may significantly influence water quality and assist in defining the optimal conditions for continuous culture of plankton. Although a substantial amount of literature is available on the nutrition and culture of common carp (Kalmer *et al.*, 1990; Keshavanath *et al.*, 2002; Manjappa *et al.*, 2002), growth of the 'Koi' ornamental variety of the common carp (*Cyprinus carpio* vr. *koi*) in organically manured culture systems have not been well documented.

The present study was designed to test the effect of cow and chicken manure on the water quality, plankton density, abundance of benthic fauna, and growth of Koi carps in outdoor cemented tanks.

Materials and Methods

The Koi carps used in this study were the hybrid of Kohaku and Showa Koi types, produced in the Hatchery Unit of Rainbow Ornamentals, Raninagar, Jalpaiguri, India. The experiments were conducted in 21 outdoor cemented tanks (capacity 2000 L) in the aforesaid fish farm. A 10 cm layer of loamy soil was placed on the bottom of each tank, which was then filled with 2000 L of groundwater 10 days prior to stocking. This interval after manure application is a prerequisite for establishing satisfactory environmental conditions for optimum zooplankton production in tanks (Jana and Chakrabarti, 1993).

Eight thousand and four hundred Koi carps, 2-3 weeks old (0.09±0.025 g), were equally distributed to each tank (400 fish / tank). The stocking density was 0.2 fish/L, commonly practiced in ornamental fish farms (Fernando and Phang, 1985). To study the effect of different application rates of cow and poultry manure on growth and survival, fish were treated for 90 days (March – May, 2002), with the seven treatments;

(1) Cowdung applied at 0.13 kg/m^3 every 10 days (C1);
(2) Cowdung applied at 0.26 kg/m^3 every 10 days (C2);
(3) Cowdung applied at 0.39 kg/m^3 every 10 days (C3);
(4) Poultry manure applied at 0.13 kg/m^3 every 10 days (P1);
(5) Poultry manure applied at 0.26 kg/m^3 every 10 days (P2);
(6) Poultry manure applied at 0.39 kg/m^3 every 10 days (P3); and
(7) A control treatment in which a commercial pelleted diet was used as feed (C).

Three tanks were randomly assigned for each treatment. The application rates of the manures correspond to 1,300-3,900 kg/ha. The high organic load was used in view of the high manuring rate (initial dose of 10,000 kg/ha and subsequent application of 5,000 kg/ha), recommended for nursery ponds in India (Jhingran, 1991). An amount of 100 L water was replaced in each tank two times a week for aeration, since most farmers in India cannot afford aeration facilities.

The amount of total nitrogen and organic carbon in the cow and poultry manures were estimated according to Micro-Kjeldahl's method (Anderson and Ingram, 1993) and Wet Oxidation method (Walkley and Black, 1934), respectively. The manures were collected from local dairy and poultry farms, and allowed to decompose for 10 days prior to application. No manure was added to the tanks in the control treatment, where a commercial pelleted feed containing 32% crude protein, 4% crude fat, 5% crude fibre, 10% crude ash, 9% moisture, and 31% nitrogen free extract was given in the amount of 5% body weight of stocked fish daily. Dry feeds were not applied to any other treatment, where the fish fed on naturally grown food.

Water samples were collected weekly at 9 A.M. from each tank in 1 L glass sampling bottles and 100 ml Winkler bottles for dissolved oxygen (DO). Collected samples were transported to a laboratory in the University of North Bengal within 2 hrs. Water quality parameters (DO, free CO_2, alkalinity, PO_4-P, NH_4-N, NO_2-N, NO_3-N, and specific conductivity) were estimated according to methods as described by APHA (1998). Temperature was recorded by a mercury thermometer. The pH was measured in situ using a portable pH meter (Hanna Instruments).

Samples of plankton were collected with a plankton net, which was made of standard bolting silk cloth (No. 21 with 77 mesh/cm^2) two times a week. Collected plankton were concentrated to 20 ml, and preserved in 4% formalin. Enumeration of 1 ml of concentrated plankton was performed under the microscope using Sedgwick Rafter counting cell. The sediments were sampled manually. Two samples, each of about 100 cm^2 area, were collected carefully with hand from each tank weekly and washed through a series of sieves ranging 260-360 μ mesh. Benthic fauna were identified and counted with the aid of a stereoscopic microscope (Macan, 1975). During the sampling utmost precaution was taken to avoid turbidity in water and an equal amount of soil was replaced in each tank precisely in the area from where sediments were collected.

The weight of the fish was recorded at the beginning of the experiment, and, every fortnightly during the culture period, five random samples of 20 fish from each tank were captured and excess water removed on paper towel through the net before the fish were individually weighed to the nearest 0.001 g. The selected fish were anaesthetized with tricaine methane sulphonate (MS-222) of 0.04 g/L concentration.

Dead fish were removed daily, they were not replaced during the course of study, and differences between the number of fish stocked and the number of fish at harvest were used to calculate percent mortality in each treatment. Fish were harvested after 90 days and weighed. The Specific Growth Rate (SGR) was calculated as:

$$SGR = 100\ [(\ln W_t - \ln W_0)/t];$$

Where W_0 and W_t are the initial and final live weight of the fish (g), respectively, and (t) is culture period in days (Ricker, 1975).

The statistical analysis were performed using MS Excel and Mstat programmes for Windows. Data from the replicates of each group were pooled for one way analysis of variance (ANOVA), and where a significant difference ($P<0.05$) was detected, a multiple comparison test (Tukey) was applied. The degree of relationship between plankton density and fish growth was determined by regression analysis and Pearson's correlation coefficient (Sunder Rao and Richard, 1999).

Results

The amount of total nitrogen in cow and poultry manures were 2.15% and 2.66%, respectively, whereas, the amount of organic carbon were 21.24% and 30.19%, respectively.

Water temperature was between 25°C and 31°C during the 90 days. The temperature did not vary

between the different treatments ($P>0.05$). The average pH values were about 7.0 in all treatments (Table 1). The DO never dropped below 5.0 mg/L during the period of the study in any treatment except C3 and P3 (Table 1). Significantly higher values of PO_4-P ($F_{6,84}>20.64$; $P<0.05$) and specific conductivity ($F_{6,84}>14.99$; $P<0.05$) were obtained in the P3 treatment than in the other treatments. Carbonate alkalinity was observed only in the treatments manured with poultry excreta (P1, P2, and P3), for very limited periods, when free CO_2 in these treatments was absent. The C3 and P3 treatments yielded significantly higher values of bicarbonate alkalinity ($F_{6,84}>11.68$; $P<0.05$), NH_4 - N ($F_{6,84}>23.27$; $P<0.05$), NO_3-N ($F_{6,84}>21.79$; $P<0.05$) and NO_2-N ($F_{6,84}>15.61$; $P<0.05$) compared to the C1, P1 and control (C) treatments (Table 1).

Examination of plankton showed that the species diversity differed considerably between the manured treatments and control. Cladocerans, which formed a substantial proportion of the total plankton composition in the manured treatments, were absent from the control treatment. Other zooplankton and phytoplankton were represented in the control with low number compared to the manured treatments (Table 2). Within the manured treatments, the abundance (no./L) of the different species varied considerably. Average zooplankton abundance was highest in P2 followed by C2, P3, C3, P1, C1, and C. The differences between treatments were significant ($F_{6,175}>1181.54$; $P<0.05$). The Copepoda was the most dominant group ranging from 52.5% of the total plankton composition in P2 to 88.74% in the control. The phytoplankton population in C3 and P3 were significantly higher ($F_{6,175}>1306.2$; $P<0.05$) than the other treatments (Table 2). The benthic fauna was represented by three groups: Oligochaeta, Diptera and Gastropoda (Table 3). The differences between treatments were significant ($F_{6,84}>207.97$; $P<0.05$). The highest values were recorded in the C3 treatment, followed in decreasing order by P3, P2, C2, P1, C1 and C treatments. *Chironomus sp.* was the most dominant benthic fauna in all the treatments (Table 3).

At harvest, maximum weight gain of Koi carps (Table 4) was achieved in the P2 treatment, followed in decreasing order by C2, P3, C3, P1, C1, and C treatments ($F_{6,14}>702.25$; $P<0.05$). The Specific Growth Rate (SGR) was quite high (>3.5) in all treatments, though the differences among the various treatments were significant ($F_{6,14}>823.71$; $P<0.05$). There was a significant difference ($F_{6,14}>31.80$; $P<0.05$) in survival of Koi carps among the treatments, ranging from 65.5% in C to 86% in P2 (Table 4).

Discussion

There was autochthonous production of plankton in all treatments, following the principle of pond fertilization. As observed from the gut analysis of common carp (Chakrabarti and Jana, 1991), zooplankton was the main source of food of the Koi carps. Zooplankton in our study was also the primary food item that is significantly correlated with the growth of Koi carps in all the seven treatments ($r = 0.957$; d.f. = 19; $P<0.05$) (Figure 1).

High application rates of cowdung and poultry manure in the C3 and P3 treatments significantly increased ($P<0.05$) the alkalinity, PO_4-P, NH_4-N, NO_2-N, and NO_3-N values of the water. NH_4-N, incorporated from organic manure application, as well as metabolism of the water body, might be considered an index of environmental stress (Jana and Chakrabarti, 1993). Jana and Barat (1984) observed an inverse relationship between NH_4-N and DO. In our experiment, lower DO values were recorded in the C3 and P3 treatments. Critical evaluation of the data revealed that the concentration of NH_4-N was inversely related to the abundance of cladocerans in the C3 ($r = -0.614$; d.f.=11; $P<0.05$) and P3 ($r = -0.688$; d.f.=11; $P<0.05$) treatments.

Table 1. Mean±SE of major water quality parameters analysed for the seven treatments. Each mean represents 13 samples collected at weekly intervals during the 3 month growth period. Different superscripts in the same row indicate statistically significant differences between means at $P<0.05$.

Parameters	Treatments						
	C1	C2	C3	P1	P2	P3	C
pH	7.32±0.10 ^{ab}	7.23±0.14 ^{ab}	7.02±0.13 ^{b}	7.49±0.11 ^{ab}	7.71±0.15 ^{a}	7.43±0.15 ^{ab}	7.62±0.11 ^{a}
Dissolved oxygen (mg/L)	6.81±0.35 ^{ab}	7.14±0.33 ^{ab}	5.23±0.27 ^{c}	6.32±0.21 ^{bc}	7.29±0.26 ^{ab}	5.14±0.24 ^{c}	7.71±0.32 ^{a}
Free CO_2 (mg/L)	4.58±0.40 ^{a}	4.08±0.25 ^{ab}	3.48±0.19 ^{ab}	2.98±0.29 ^{b}	2.96±0.45 ^{b}	3.15±0.41 ^{b}	3.09±0.19 ^{b}
CO_3 alkalinity (mg/L)	-	-	-	0.65±0.65	1.32±0.89	0.74±0.74	-
HCO_3 alkalinity (mg/L)	55.08±2.38 ^{cd}	75.69±4.53 ^{bcd}	93.54±7.93 ^{ab}	64.73±4.05 ^{cd}	78.19±5.57 ^{bc}	106.23±9.28 ^{a}	53.15±1.74 ^{d}
PO_4 – P (mg/L)	0.152±0.016 ^{cd}	0.271±0.024 ^{bc}	0.328±0.038 ^{ab}	0.161±0.014 ^{c}	0.301±0.028 ^{ab}	0.410±0.044 ^{a}	0.029±0.002 ^{d}
NH_4 – N (mg/L)	0.129±0.014 ^{de}	0.181±0.022 ^{cd}	0.400±0.044 ^{ab}	0.287±0.030 ^{bc}	0.321±0.030 ^{ab}	0.429±0.041 ^{a}	0.03±0.002 ^{e}
NO_2 – N (mg/L)	0.026±0.003 ^{bc}	0.032±0.003 ^{bc}	0.046±0.004 ^{a}	0.023±0.003 ^{cd}	0.039±0.003 ^{ab}	0.053±0.004 ^{a}	0.008±0.001 ^{d}
NO_3 – N (mg/L)	0.121±0.011 ^{d}	0.30±0.031 ^{bc}	0.412±0.044 ^{ab}	0.162±0.019 ^{cd}	0.329±0.031 ^{b}	0.50±0.061 ^{a}	0.03±0.005 ^{d}
Specific conductivity (mmhos/cm)	0.431±0.023 ^{cd}	0.572±0.043 ^{bc}	0.688±0.054 ^{b}	0.412±0.025 ^{cd}	0.68±0.056 ^{b}	1.151±0.665 ^{a}	0.272±0.007 ^{d}

Table 2. Species composition, abundance (no./L) and relative abundance (% of total numbers) of plankton in culture tanks manured with cowdung and poultry excreta at different rates, and Control. Each mean value represents data from 26 samples collected two times a week during the 13 week (3 month) growth period.

Species	C1		C2		C3		P1		P2		P3		C	
	(no./L)	(%)	(no./L)	(%)	(no./L)	(%)	(no./L)	(%)	(no./L)	(%)	(no./L)	(%)	(no./L)	(%)
Daphnia sp.	72.31	11.97	150.20	12.59	60.12	6.50	118.70	14.75	212.39	14.15	71.19	7.04	-	-
Moina sp.	81.20	13.44	191.66	16.07	123.20	13.31	120.12	14.93	254.12	16.93	126.12	12.48	-	-
Bosmina sp.	18.24	3.02	26.42	2.22	28.20	3.05	21.30	2.65	31.24	2.08	28.24	2.79	-	-
Cladocera	171.75	28.43	368.28	30.88	211.52	22.86	260.12	32.34	497.75	33.16	225.55	22.31	-	-
Cyclops sp.	180.12	29.81	310.21	26.01	251.50	27.18	214.29	26.64	386.52	25.75	292.55	28.94	152.14	49.04
Diaptomus sp.	118.36	19.59	260.24	21.82	182.12	19.68	164.12	20.41	290.24	19.34	198.21	19.61	80.06	25.80
Nauplii	50.24	8.32	94.26	7.90	62.24	6.73	58.13	7.23	112.12	7.47	62.45	6.18	43.12	13.90
Copepoda	348.72	57.72	664.71	55.73	495.86	53.58	436.54	54.28	788.88	52.56	553.21	54.72	275.32	88.74
Brachionus sp.	28.23	4.67	46.12	3.87	36.28	3.92	30.12	3.74	58.19	3.88	45.12	4.46	20.33	6.55
Keratella sp.	12.17	2.02	24.33	2.03	28.20	3.05	29.30	3.64	38.16	2.54	26.16	2.59	-	-
Rotifera	40.40	6.69	70.45	5.90	64.48	6.97	59.42	7.38	96.35	6.42	71.28	7.05	20.33	6.55
Chlorella sp.	6.24	1.03	8.71	0.73	24.96	2.70	6.11	0.76	16.88	1.12	26.60	2.63	-	-
Navicula sp.	16.79	2.78	30.82	2.58	42.14	4.55	17.30	2.15	42.51	2.83	54.38	5.38	4.24	1.37
Spirogyra sp.	9.02	1.49	11.35	0.95	27.90	3.01	7.15	0.89	12.65	0.84	28.20	2.79	4.22	1.36
Scenedesmus sp.	-	-	2.25	0.19	9.12	0.98	2.04	0.25	3.07	0.20	6.59	0.65	-	-
Phacus sp.	11.26	1.86	33.16	2.78	45.26	4.89	13.73	1.71	39.51	2.63	41.13	4.07	4.02	1.30
Synedra sp.	-	-	2.98	0.25	4.19	0.45	1.90	0.24	3.28	0.22	4.02	0.40	2.12	0.68
Phytoplankton	43.31	7.17	89.27	7.48	153.57	16.59	48.23	6.0	117.90	7.86	160.92	15.92	14.60	4.71
Total plankton	604.18		1192.71		925.43		804.31		1500.88		1010.96		310.25	

Table 3. Species composition and abundance (no./100 cm^2) of benthic fauna in culture tanks manured with cowdung and poultry excreta at different rates, and Control. Each mean value of represents the data from 13 samples collected weekly during the 3 month growth period.

Species	Treatments						
	C1 (no./100 cm^2)	C2 (no./100 cm^2)	C3 (no./100 cm^2)	P1 (no./100 cm^2)	P2 (no./100 cm^2)	P3 (no./100 cm^2)	C (no./100 cm^2)
Tubifex sp.	2.92	5.92	5.53	4.61	5.76	5.38	1.53
Chaetogaster sp.	1.46	2.46	2.53	1.23	3.15	3.53	0.75
Oligochaeta	4.38	8.38	8.06	5.84	8.91	8.91	2.29
Chironomus sp.	6.76	7.69	10.69	6.30	7.38	9.23	2.35
Diptera	6.76	7.69	10.69	6.30	7.38	9.23	2.38
Pila sp.	1.92	1.53	1.84	2.00	2.07	1.77	1.76
Lymnaea sp.	1.84	1.92	2.23	1.23	1.46	1.84	1.53
Gastropoda	3.76	3.45	4.07	3.23	3.53	3.61	3.29
Total benthic fauna	14.90	19.52	22.82	15.37	19.82	21.75	7.96

Table 4. Mean±SE harvest weight, weight gain, specific growth rate (SGR) and survival rate at the end of 13 week growth period (March – May, 2002) of Koi carps reared in concrete tanks manured with cowdung and poultry excreta, applied at different rates and Control

	Treatments						
	C1	C2	C3	P1	P2	P3	C
Manure Application rate (kg/m^3/10 days)	0.13 (Cowdung)	0.26 (Cowdung)	0.39 (Cowdung)	0.13 (Poultry excreta)	0.26 (Poultry excreta)	0.39 (Poultry excreta)	-
Harvest weight (g)	3.31 ± 0.05^{f}	7.47 ± 0.12^{b}	4.89 ± 0.13^{d}	4.28 ± 0.10^{e}	9.17 ± 0.07^{a}	6.47 ± 0.11^{c}	3.03 ± 0.04^{f}
Weight gain (g)	3.22 ± 0.05^{f}	7.38 ± 0.12^{b}	4.80 ± 0.13^{d}	4.19 ± 0.10^{e}	9.08 ± 0.07^{a}	6.38 ± 0.11^{c}	2.94 ± 0.04^{g}
SGR (%/day)	4.01 ± 0.07^{f}	4.93 ± 0.08^{b}	4.44 ± 0.10^{d}	4.29 ± 0.06^{e}	5.14 ± 0.03^{a}	4.76 ± 0.04^{c}	3.94 ± 0.02^{g}
Survival rate (%)	70.5 ± 0.56^{bc}	81.5 ± 0.24^{a}	70.25 ± 0.49^{bc}	71.75 ± 0.80^{b}	86.0 ± 0.32^{a}	70.25 ± 0.28^{bc}	65.5 ± 0.37^{c}
Growth equation*	$Y=e^{-1.966+0.247T}$	$Y=e^{-0.551+0.220T}$	$Y=e^{-1.261+0.220T}$	$Y=e^{-1.758+0.241T}$	$Y=e^{-0.468+0.211T}$	$Y=e^{-1.017+0.222T}$	$Y=e^{-2.336+0.266T}$

* The growth models predict mass of fish (Y = g fish) as a function of time (T = weeks).
Different superscripts indicate statistically significant differences between means at $P<0.05$.

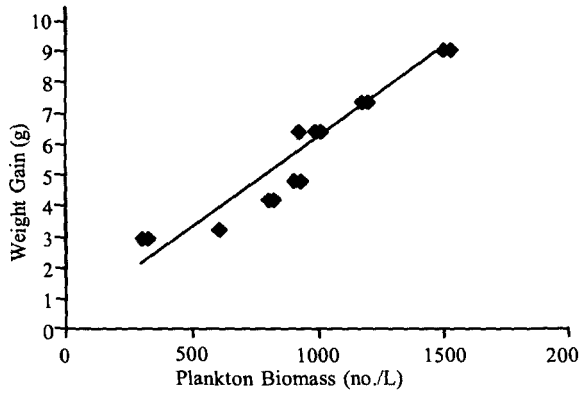

Figure 1. Relationship between weight gain of Koi carp and plankton biomass in the seven treatments.

Differences in the relative abundance of some groups of zooplankton might have contributed to the differential growth responses of the Koi carps. Lower weight gain, SGR and survival rate of Koi carp in the control treatment may be attributed to the insufficient quantity of zooplankton in the system (Szlaminska and Przybye, 1986). Zoobenthos form essential food items of many cultivated fishes in Indian ponds (Jhingran, 1991). However, in our study, the benthic fauna population (Table 3) was low and seemed insufficient to sustain fish production by itself. Compared to larger pond environment, depleted zoobenthos production have been reported in culture tanks (Majumder *et al.*, 2002). Although gut content analysis of the Koi carps were not carried out in our study, it can be assumed that the zoobenthos, with its small population played a secondary role to plankton as food for the carp. Statistical analysis of the data revealed significantly higher concentration of benthic fauna ($P<0.05$) in C3 and P3 compared to the other treatments. This may be influenced by higher rate of manure application (Wade and Stirling, 1999).

It is well known that high yield of fish can be achieved by higher abundance of plankton in culture system. However, it is not possible to increase the application rate of organic manures after a certain limit because this may reduce water quality, which cause stress for reproduction of essential zooplankton thereby causing adverse effect on fish growth. Studies on life history parameters of *Daphnia* sp. and *Moina* sp. (Jana and Chakrabarti, 1993) suggest that growth, reproductive potentials, and longevity of each species are affected by the nutrient conditions of the culture media. Dhawan and Kaur (2002) reported a decrease in cladoceran population with increased organic manure application in ponds. The presence of relatively higher density of zooplankton in C2 and P2 compared to C3 and P3 could be a consequence of relatively suitable environment in terms of water quality and food abundance (Jana and Chakrabarti, 1997). As a result, the weight gain of Koi carps were significantly higher ($P<0.05$) in the C2 and P2 treatments, compared to the C3 and P3 treatments, respectively. Similar decline in plankton biomass due to undesirable water quality with very high amounts of fertilizers have been reported by many authors (Lin *et al.*, 1997; Garg and Bhatnagar, 2000; Azim *et al.*, 2001; Cheikyula *et al.*, 2001). Perhaps, the significantly higher level of nutrients and low dissolved oxygen in the C3 and P3 treatments lowered the grazing activity by carp in these two treatments, compared to the C2 and P2 treatments, respectively. Again, the differences in the weight gain of Koi carps observed among the different treatments were not essentially due to changes in the water quality, since, growth in the C1 and P1 treatments were significantly lower than C2 and P2 treatments, respectively, despite having good water quality. It might well be that the weight gain is more directly related to differences in food concentrations, although the zooplankton density and water quality were closely related to each other.

In any given application rate, the poultry manure appeared to be more effective compared to cowdung (Table 4). In the present investigation, an application rate of 0.26 kg/m^3 every 10 days appeared to be the most suitable for Koi carp tanks manured with cowdung and poultry excreta. Higher application rates reduce water quality, deplete the plankton population and cause adverse impact on the growth of the Koi carp. Further research on husbandry management of Koi carp needs to be conducted.

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EFFECT OF WATER EXCHANGE ON WATER QUALITY AND THE PRODUCTION OF ORNAMENTAL CARP (*CYPRINUS CARPIO* VAR. *KOI* L.) CULTURED IN CONCRETE TANKS MANURED WITH POULTRY EXCRETA

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ABSTRACT. The effect of water exchange on water quality, plankton abundance, and the production of koi carp, *Cyprinus carpio* var. *koi* L., cultured in outdoor concrete tanks manured with poultry excreta was determined. Individual weight gain and survival rates of fish (initial weight 0.09 ± 0.02 g) were compared among five culture regimes, where a volume of 100 dm^3 water was exchanged: (1) every day (WE1); (2) every alternate day (WE2); (3) two times a week (WE3); (4) once a week (WE4); and (5) a control treatment with no water exchange (NE). Significantly higher concentrations ($P < 0.05$) of conductivity, NH_4-N, NO_2-N, NO_3-N, PO_4-P and bicarbonate alkalinity were recorded in the NE treatment. Plankton volume was highest in WE1 ($P < 0.05$). The weight gain and number of koi carp of marketable size were significantly higher ($P < 0.05$) in WE1. There was a significant difference in the survival of koi carp among the treatments ranging from 60.43% (NE) to 95.21% (WE1). The results suggest a water exchange of 100 dm^3 daily (WE1) was the most effective for koi carp tanks manured with poultry excreta as better water quality and greater plankton abundance were both maintained in the system.

Key words: KOI CARP (*CYPRINUS CARPIO* VAR. *KOI*), WATER EXCHANGE, WATER QUALITY, PLANKTON ABUNDANCE, FISH PRODUCTION

INTRODUCTION

Production of animals for the aquarium hobbyist trade is growing rapidly. The annual global trade has increased from US$ 4.5 billion in 1995 to about US$ 9 billion in 2002 (Swain and Jena 2002). Establishing an ornamental fish culture industry has long been felt to be one means to diversify the aquaculture sector in India.

In the case of food fish culture, the intensification of natural production is stimulated through fertilization or organic manuring in ponds (Moore 1985). Since biological

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productivity is often limited by nutrients, pond fertilization is of great importance to supplement nutrient deficiency and augment aquatic productivity through autotrophic and heterotrophic pathways (Green et al. 1989, Schroeder et al. 1990, Knud-Hansen and Batterson 1994). However, pond fertilization using high amounts of manure can lead to water quality deterioration, including the severe depletion of dissolved oxygen, high biological and chemical oxygen demand, and high ammonia levels (Boyd 1982). Primary production in excessively fertilized ponds can limit light penetration (Hepher 1962). The resultant stress can ultimately lead to exhaustion, disease, and mortality in fish (Francis-Floyd 1990). Moreover, ornamental fish, unlike food fish, are sold by number and have to be visually attractive to be accepted in the market, and stressed fish may be aesthetically unattractive to potential customers. In light of these factors, particular pond management techniques need to be developed to create the best environment for the fish, while utilizing animal wastes that can sustain productivity at low cost.

Fertilization with manure with water exchange have proved to be more effective than manured systems without water exchange in maintaining better water quality and lower mortality rates in common carp, *Cyprinus carpio* L. (Chakrabarti and Jana 1990), Indian major carp rohu, *Labeo rohita* (Ham.), and mrigal, *Cirrhinus mrigala* (Ham.) (Chakrabarti and Jana 1998). The water quality management in the culture of ornamental fishes in Indian conditions remains to be elaborated.

The present study was conducted to compare the effect of different water exchange regimes on the growth and survival of ornamental fish in manured culture systems. Koi carp, *Cyprinus carpio* var. *koi* L., were used as a model species and poultry excreta was applied as a standard organic manure.

MATERIALS AND METHODS

ANIMALS

A total of 6000 two-week old koi carp larvae were obtained from a local fish farm (Rainbow Ornamentals, Raninagar, Jalpaiguri, India). The koi carp were the offspring of a mixed commercial production by 25 pairs of parents of the Kohaku, Bekko, and Asagi koi types. After a one-week acclimatization period, the koi carp of an initial weight of 0.09 ± 0.02 g (average BW ± SD; N = 50) were evenly distributed

in 15 outdoor concrete tanks (capacity 2000 dm^3). The stocking density corresponded to 0.2 fish dm^{-3}, which is widely practiced in ornamental fish farms (Fernando and Phang 1985). All the tanks were manured every 10 days with poultry excreta applied at 0.26 kg m^{-3}, as standardized in an earlier experiment (Jha et al. 2004).

EXPERIMENTAL PROCEDURE

A 10 cm layer of soil was placed at the bottom of each tank, which was then filled with 2000 dm^3 of groundwater 10 days prior to stocking. A 3.175 cm GI pipe was fitted to the outlet of each tank in such a way that water in excess of 2000 dm^3 would automatically flow out. A plankton net (No. 21 with 77 mesh cm^{-2}) bordered the outlet preventing escape of plankton with the outflowing water. Five experimental groups were cultured, each in triplicate, for 90 days (June-August 2002): (1) 100 dm^3 water exchange once daily (WE1); (2) 100 dm^3 water exchange every alternate day (WE2); (3) 100 dm^3 water exchange two times a week (WE3); (4) 100 dm^3 water exchange once a week (WE4); and (5) a treatment without any water exchange (NE). A single layer of plastic bird netting was used to cover the tanks.

DATA COLLECTION

The amounts of total nitrogen and organic carbon in the poultry manure were estimated according to the Micro-Kjeldahl (Anderson and Ingram 1993) and Wet Oxidation methods (Walkley and Black 1934), respectively.

Water samples were collected weekly. Water quality parameters (dissolved oxygen, BOD, free carbon dioxide, total alkalinity, conductivity, ammonium, nitrite, nitrate, and phosphate) were estimated according to methods described by APHA (1998). pH was measured *in situ* using a portable pH meter (Hanna Instruments, Rua do Pindelo, Portugal). Temperature was recorded with a centigrade thermometer.

Samples of plankton were collected with a plankton net made of standard bolting silk cloth (No. 21) two times a week. The collected plankton samples were concentrated to 20 cm^3, preserved in 4% formalin, and counted under a stereoscopic microscope using a Sedgwick Rafter Counting Cell.

The body weight of the fish was recorded at the beginning of the experiment. At fortnightly intervals during the culture period, five random samples of 20 fish from each tank were netted and excess water removed on paper toweling through the net

before the fish were weighed individually to the nearest ± 1 mg. For this, the fish were anaesthetized with tricaine methane sulphonate (MS-222) at a concentration of 0.04 g dm^{-3}. Dead fish were removed daily, they were not replaced during the course of study, and differences between the number of fish stocked and the number of fish at harvest were used to calculate the percentage of mortality in each treatment. Results in percentage were normalized using angular transformation (Sokal and Rohlf 1969) before being subjected to further statistical analysis. The fish were harvested after 90 days and weighed. The Specific Growth Rate (SGR) was calculated as: $SGR = 100 [(\ln W_t - \ln W_0) t^{-1}]$; where W_0 and W_t are the initial and final body weight of the fish (g), respectively, and (t) is the culture period in days (Ricker 1975).

STATISTICAL ANALYSIS

The data on body weights, SGR, and survival rates were compared using one-way analysis of variance (ANOVA) and Tukey's HSD test (Zar 1996). The differences were considered statistically significant at the probability level of $P \leq 0.05$. The degree of linear relationship between plankton density and fish growth was determined by means of correlation coefficients following Karl Pearson's method (SunderRao and Richard 1999).

The number of marketable fish at the end of growth period was calculated using the function for a normal distribution curve, where $z = (y-\mu) \sigma^{-1}$; y is the least marketable weight (g), μ is the mean weight of the population, σ is the standard deviation of the total weight and z follows the standard normal probability distribution which determines the probability of finding fish above a given range. The number of marketable fish (n) was then determined using the table value of the normal probability distribution (P) as follows: n = (1-P) × total number of fish produced.

RESULTS

The amounts of total nitrogen and organic carbon in the poultry manure were 2.66% and 30.19%, respectively. Water temperature ranged between 24 and 35°C during the investigation period, whereas pH varied from 6.7-8.6 (Table 1). Higher water exchange rates increased the average dissolved oxygen in WE1 (7.94 mg dm^{-3}), which was significantly higher ($P < 0.05$) than the other treatments. In contrast, significantly higher ($P < 0.05$) BOD values were obtained in NE (4.63 mg dm^{-3}).

The NE treatment showed the highest concentrations of conductivity, NH_4-N, NO_2-N, NO_3-N, PO_4-P, and bicarbonate alkalinity, which were significantly higher ($P < 0.05$) than the other treatments (Table 1). Carbonate alkalinity was observed only in the WE1, WE2, and WE3 treatments, for very limited periods, when the free CO_2 content in these treatments was absent. Within the different treatments with water exchange (WE1, WE2, WE3, and WE4), there were no significant differences ($P > 0.05$) between the average values of bicarbonate alkalinity, NH_4-N, NO_2-N, and specific conductivity (Table 1).

TABLE 1

Mean ± SE of the major water quality parameters analyzed for the five treatments at weekly intervals during the three-month growth period. Data in the same row with different superscripts are significantly different ($P < 0.05$)

Parameters	Treatment				
	WE1	WE2	WE3	WE4	NE
pH*	6.8 - 8.6	6.8 - 8.6	6.8 - 8.5	6.5 - 8.4	6.2 - 8.2
Dissolved oxygen (mg dm^{-3})	7.94 ± 0.32^{a}	7.37 ± 0.30^{ab}	6.91 ± 0.24^{bc}	6.08 ± 0.18^{c}	5.08 ± 0.26^{d}
Biological Oxygen Demand (mg dm^{-3})	1.23 ± 0.17^{c}	1.46 ± 0.23^{bc}	1.99 ± 0.36^{bc}	2.62 ± 0.28^{b}	4.63 ± 0.52^{a}
Free CO_2 (mg dm^{-3})	0.42 ± 0.15^{c}	0.49 ± 0.17^{c}	0.61 ± 0.20^{c}	1.86 ± 0.23^{b}	3.16 ± 0.46^{a}
CO_3 alkalinity (mg dm^{-3})	0.66 ± 0.17	0.60 ± 0.19	0.13 ± 0.13	-	-
HCO_3 alkalinity (mg dm^{-3})	95.19 ± 6.89^{b}	102.95 ± 8.42^{b}	110.07 ± 9.66^{ab}	115.5 ± 9.38^{ab}	146.25 ± 11.02^{a}
PO_4 -P (mg dm^{-3})	0.226 ± 0.028^{b}	0.253 ± 0.031^{b}	0.294 ± 0.035^{b}	0.428 ± 0.061^{ab}	0.563 ± 0.080^{a}
NH_4 -N (mg dm^{-3})	0.163 ± 0.021^{b}	0.183 ± 0.025^{b}	0.202 ± 0.027^{b}	0.308 ± 0.044^{b}	0.753 ± 0.148^{a}
NO_2 -N (mg dm^{-3})	0.021 ± 0.005^{b}	0.026 ± 0.005^{b}	0.027 ± 0.006^{b}	0.038 ± 0.010^{b}	0.211 ± 0.048^{a}
NO_3 -N (mg dm^{-3})	0.187 ± 0.022^{b}	0.217 ± 0.028^{b}	0.272 ± 0.033^{b}	0.390 ± 0.056^{ab}	0.608 ± 0.096^{a}
Specific Conductivity (m mhos cm^{-1})	0.586 ± 0.051^{b}	0.612 ± 0.053^{b}	0.672 ± 0.065^{b}	0.786 ± 0.072^{b}	1.156 ± 0.146^{a}

** for pH, the range of recorded values are presented*

On average, plankton volume was highest in the WE1 treatment followed in descending order by the WE2, WE3, WE4, and NE treatments ($P < 0.05$) (Table 2). The plankton volume primarily consisted of zooplankton. Phytoplankton accounted for 9.53% (WE1) to 46.08% (NE) of the total plankton content. The abundance (no. dm^{-3}) of the different plankton groups also differed considerably. The average number of cladocerans in WE1 (597.09) was 462% the average number of the same group in the NE treatment (129.17). The copepoda was most dominant among the zooplankton in all the treatments ranging from 47.65 % in WE1 to 53.91 % in NE (Table 2).

TABLE 2

Species composition, abundance (no. dm^{-3}), and relative abundance (% of total numbers) of plankton in culture tanks manured with poultry excreta under different water exchange regimes. Each mean value represents data from 25 samples collected two times a week during the three-month growth period

Species	Treatment									
	WE1		WE2		WE3		WE4		NE	
	(no. dm^{-3})	(%)	(no. dm^{-3})	(%)	(no. dm^{-3})	(%)	(no. dm^{-3})	(%)	(no. dm^{-3})	(%)
Chlorella sp.	30.12	2.01	29.12	2.06	26.24	2.14	40.16	4.43	52.12	8.14
Navicula sp.	38.24	2.55	42.12	2.98	40.76	3.33	43.71	4.82	56.11	8.76
Spirogyra sp.	25.12	1.67	23.06	1.63	31.12	2.54	51.16	5.64	68.24	10.66
Scenedesmus sp.	8.70	0.58	8.12	0.57	9.22	0.75	16.44	1.81	28.18	4.40
Phacus sp.	36.15	2.41	42.26	2.99	48.12	3.93	64.14	7.07	80.15	12.52
Synedra sp.	4.61	0.30	5.03	0.35	5.62	0.46	6.78	0.74	10.20	1.59
Total phytoplankton	142.94	9.53	149.71	10.59	161.08	13.17	222.39	24.52	295.00	46.08
Daphnia sp.	252.30	16.82	200.72	14.20	178.66	14.61	91.29	10.06	39.12	6.11
Moina sp.	276.24	18.41	258.22	18.27	194.52	15.91	128.24	14.14	66.05	10.32
Bosmina sp.	68.55	4.57	63.32	4.48	63.20	5.17	50.14	5.53	24.00	3.75
Cladocera	597.09	39.80	522.26	36.96	436.38	35.69	269.67	29.73	129.17	20.18
Cyclops sp.	324.05	21.60	368.24	26.06	272.14	22.26	184.62	20.36	95.12	14.86
Diaptomus sp.	252.44	16.83	205.12	14.52	196.02	16.03	126.55	13.95	60.28	9.42
Nauplii	70.12	4.67	66.24	4.69	66.16	5.41	44.28	4.88	22.14	3.46
Copepoda	646.61	43.10	639.60	45.27	534.32	43.70	355.45	39.19	177.54	27.73
Brachionus sp.	67.08	4.47	60.92	4.31	56.12	4.59	32.14	3.54	20.18	3.15
Keratella sp.	46.22	3.08	40.32	2.85	34.66	2.83	27.20	3.00	18.22	2.84
Rotifera	113.30	7.55	101.24	7.16	90.78	7.42	59.34	6.54	38.40	5.99
Total zooplankton	1357.00	90.47	1263.10	89.41	1061.48	86.83	684.46	75.48	345.11	53.92
Total plankton	1499.94		1412.81		1222.56		906.85		640.11	

The final body weight of the koi carp ranged from 3.01 to 9.56 g in the different treatments (Table 3). At harvest, maximum weight gain was achieved in the WE1 treatment, followed in descending order by the WE2, WE3, WE4, and NE treatments ($P < 0.05$). There was a direct correlation ($r = 0.986$; d.f.= 13; $P < 0.01$) between the weight gain of koi carp and the amount of plankton present in the five treatments (Fig. 1). All the data related to regressions of the natural log of average fish weight over time for each treatment fitted an exponential model with R^2 values of 0.729, 0.717, 0.739, 0.882, and 0.903 for the WE1, WE2, WE3, WE4, and NE treatments, respectively (Table 3).

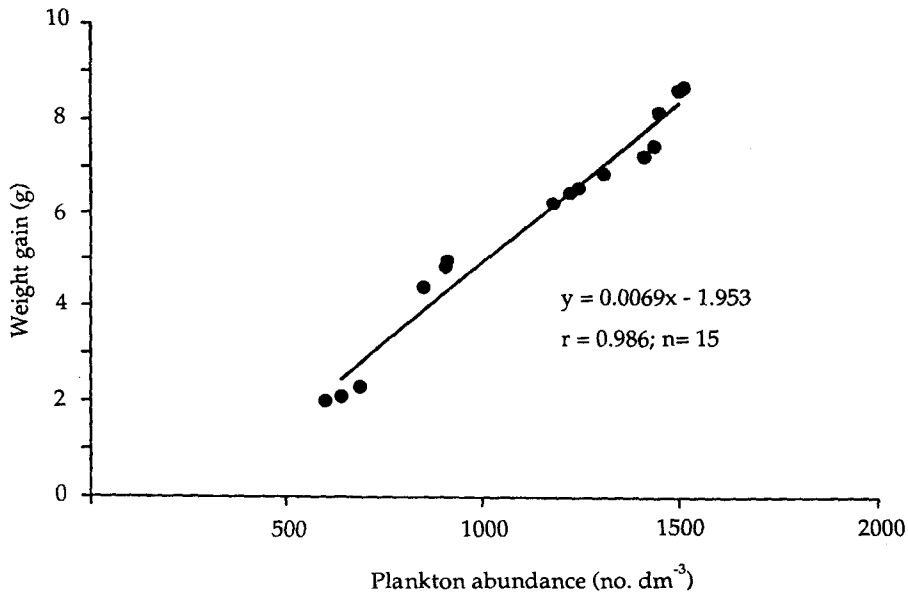

Fig. 1. Relationship between weight gain of koi carp and plankton abundance in the five treatments.

TABLE 3

Mean ± SE of fish growth parameters at the end of the three-month growth period (June - August, 2002) of koi carp reared in concrete tanks manured with poultry excreta under different water exchange regimes. Data in the same row with different superscripts are significantly different (P < 0.05)

	Treatment				
	WE1	WE2	WE3	WE4	NE
Initial body weight (g)	0.09 ± 0.02^{a}	0.09 ± 0.02^{a}	0.09 ± 0.02^{a}	0.09 ± 0.02^{a}	0.09 ± 0.02^{a}
Harvest weight (g)	9.56 ± 0.08^{a}	8.18 ± 0.08^{b}	7.39 ± 0.10^{b}	5.75 ± 0.08^{c}	3.01 ± 0.11^{d}
Weight gain (g)	9.47 ± 0.08^{a}	8.09 ± 0.08^{b}	7.30 ± 0.10^{b}	5.66 ± 0.08^{c}	2.91 ± 0.11^{d}
SGR (% day^{-1})	5.16 ± 0.05^{a}	5.08 ± 0.06^{b}	4.89 ± 0.12^{c}	4.61 ± 0.08^{d}	3.92 ± 0.03^{e}
Survival rate (%)	95.21 ± 1.03^{a}	89.61 ± 0.76^{b}	81.96 ± 0.89^{c}	74.84 ± 0.34^{d}	60.43 ± 2.39^{e}
Growth Equation*	$Y = e^{-1.56+0.320t}$	$Y = e^{-1.55+0.309t}$	$Y = e^{-1.65+0.306t}$	$Y = e^{-1.81+0.292t}$	$Y = e^{-2.04+0.262t}$
	(R^2 = 0.729)	(R^2 = 0.717)	(R^2 = 0.739)	(R^2 = 0.882)	(R^2 = 0.903)

**The growth models predict weight of fish (Y = g fish) as a function of time (t = weeks)*

The Specific Growth Rate (SGR) was quite high (> 3.5) in all the treatments, although the differences among the various treatments were significant (P < 0.05). There was a significant difference (P < 0.05) in the survival of koi carp among the treatments, ranging from 60.43% (NE) to 95.21% (WE1). To determine the output of marketable fish, the percentage and number of fish exceeding a total weight of 4 g was

estimated from the size-frequency distribution at the end of the study. The number of marketable fish was significantly higher in WE1 ($P < 0.05$), followed in descending order by the WE2, WE3, WE4, and NE treatments (Table 4).

TABLE 4

The average number of marketable fish (those heavier than 4.0 g) produced, together with marketable fish produced expressed as a percentage of total number of fish produced (A) and as a percentage of number of fish stocked (B) in the five treatments

Treatment	Number of marketable fish produced (fish tank^{-1}) *	Marketable fish (%)	
		A	B
WE1	380 ^{a}	100	95.21
WE2	358 ^{b}	100	89.61
WE3	327 ^{c}	100	81.75
WE4	300 ^{d}	100	74.90
NE	0.05 ^{e}	0.020	0.012

** Different superscripts in this column represent statistically significant differences ($P<0.05$)*

DISCUSSION

There was autochthonous production of plankton in all the treatments, following the principal of pond fertilization. As observed from the gut analysis of common carp (Chakrabarti and Jana 1991), zooplankton formed the main source of food. Water exchange rates had a direct influence on the water quality in the different treatments. The lack of water exchange in the NE treatment significantly lowered the dissolved oxygen ($P < 0.05$) and simultaneously increased specific conductivity, PO_4-P, NH_4-N, NO_2-N, NO_3-N, and BOD, compared to the other treatments (Table 1). According to Pechar (2000), the gradual accumulation of organic matter in a water body leads to the subsequent dominance of biodegradation and decomposition processes and causes an oxygen deficit. The resulting release of nutrients leads to excessive levels of autotrophic production, as well as changes in the species composition of plankton. Water quality deteriorates through the dynamic, multiple feedback process.

NH_4-N, incorporated from the application of organic manure as well as the metabolism of the water body, might be considered an index of environmental stress (Jana and Chakrabarti 1993). High concentrations of NH_4-N were found to restrict the occurrence of many small protozoans like ciliates that are considered as excellent food for cladocerans (Pfister et al. 2002). The presence of a relatively higher density of cladocerans in

the WE1, WE2, WE3, and WE4 treatments, compared to NE, might be a consequence of a better environment in terms of water quality and food abundance. According to Herbert (1978), the maximum size reached by individuals of a particular species of Daphnia depends upon the food supply. Studies on life history parameters of *Daphnia* sp. (Jana and Pal 1983, Murugan 1989) and *Moina* sp. (Jana and Pal 1985) also suggest that growth, reproductive potential, and the longevity of each species are affected by the nutrient conditions of the culture media.

Perhaps the significantly high level of nutrients and BOD, along with low dissolved oxygen concentration in the NE treatment, lowered the grazing activity of the carp. The results clearly indicate that no water exchange in the NE treatment yielded the lowest number ($P < 0.05$) of saleable fish. In contrast to food fish production, where the total number of fish produced determines productivity, ornamental fish can only be sold once they have reached a particular size. The systematic discharge of water in WE1, WE2, WE3, and WE4 treatments significantly increased ($P < 0.05$) the number of marketable fish (Table 4). The greater dilution of the manure in these four treatments improved water quality (Table 1) and caused greater plankton abundance (Table 2) compared to the NE treatment, although the plankton volume within the four water exchange regimes differed significantly ($P < 0.05$). Differences in the relative abundance of some groups of zooplankton might have contributed to the differential growth responses and the survival of the carp. The unavailability or a non-continuous supply of preferred food have been reported to influence cannibalism in koi carp larvae (Appelbaum et al. 1986, Van Damme et al. 1989). Rothbard (1982) reported low survival rates in common carp as a result of severe competition for food when stocked at high densities. Interestingly, reduction in cannibalism in common carp was demonstrated by Von Lukowicz (1979), when a continuous supply of live food was maintained. Food availability is probably the most important factor determining the cannibalism rate in fish larvae (Hecht and Appelbaum 1988). As reported in this paper, the influence of plankton level on the growth heterogeneity and survival rate (Table 3) of koi carp larvae supports this last hypothesis.

The survival rate of koi carp was also influenced by water quality. Nitrite ions are toxic to fish, causing methaemoglobinemia (Tomasso et al. 1979). It is present in water as an intermediate in the bacterial oxidation of ammonia, the major nitrogenous waste product of fish, to nitrate (Das et al. 2004). An increase in the nitrite content in water exerts considerable stress on the fish resulting in growth suppression, tissue damage,

and mortality (Lewis and Morris 1986) resulting in poor biomass production. Diminished respiration ability in nitrite-exposed grass carp, *Ctenopharyngodon idella* (Val.), was reported by Alcaraz and Espina (1997). Korwin-Kossakowski and Ostaszewska (2003) also reported the adverse impact nitrite exposure had on common carp respiration and growth. Allowable levels are therefore low; NO_2-N levels above 0.06 mg dm^{-3} have been observed to cause a minimal degree of harm in rainbow trout, *Oncorhynchus mykiss* (Walbaum), after three weeks of exposure (Wedemeyer and Yasutake 1978). The need to draw on such data arises from the relative absence of data on ornamental fishes. During the current experiment, koi carp larvae were exposed to an average NO_2-N concentration of 0.211 mg dm^{-3} in NE for three months, which was higher than the 0.06 mg dm^{-3} limit reported for rainbow trout. Compared to NO_2-N, NO_3-N is relatively harmless and can cause stress only at very high levels (Asano et al. 2003).

Unionized ammonia is also regarded as highly poisonous to fish (Arillo et al. 1981). The permeability of the uncharged and lipid soluble unionized ammonia (NH_3) to plasma membranes is higher compared with the ionized form, and therefore it is considered to be the more toxic form (Meade 1985). Earlier studies have shown that common carp are relatively sensitive to unionized ammonia with a reported LC_{50} value of 0.44-1.9 mg dm^{-3} (Dabrowska and Sikora 1986, Xu et al. 1994). Although unionized ammonia was not measured in the current experiment, it can be assumed that high temperature and pH levels during the entire growth period would block the ionizing process of NH_3 to the relatively non-toxic NH_4-N (Ng et al. 1992). In the current study, the average NH_4-N in NE was 0.753 mg dm^{-3}, when the average pH was 7.36 and the average temperature was above 30°C. Under these conditions, the percentage of NH_3 in the water was estimated to be about 2% of the NH_4-N (Emerson et al. 1975), *i.e.*, 0.015 mg dm^{-3}, which is below the threshold limit of 0.44 mg dm^{-3}. However, according to Parma de Croux and Loteste (2004), even an incidental increase in the pH to more than 8.0 in such a situation could lead to high mortality due to a significant increase in NH_3 toxicity. Mortality might also arise due to depressions of feeding when water quality is sub-standard (Asano et al. 2003). These factors probably influenced the low survival rate (60.43%) of koi carp in the NE treatment.

The continuous supply of oxygen through aeration is known to promote nitrification in ponds, thereby lowering ammonia levels (Avnimelech et al. 1986). Since most farmers in India cannot afford aeration equipment, water exchange is used as an alter-

native to maintain water quality. However, high levels of water exchange could flush out nitrifying bacteria leading to reduced nitrification and increased ammonia concentrations (Diab et al. 1992, Milstein et al. 2001). Perhaps the solution lies in a low level of water exchange (only 5%, as in the present study) but with increased frequency. From the present investigation, a daily water exchange rate of 100 dm^3 (WE1) appeared to be the most effective for koi carp tanks manured with poultry excreta. No water exchange (NE) resulted in water quality deterioration, the depletion of the plankton population, and an adverse impact on fish growth.

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STRESZCZENIE

WPŁYW CZĘSTOTLIWOŚCI WYMIANY WODY NA JEJ JAKOŚĆ I PRODUKCJĘ KARPIA KOI (*CYPRINUS CARPIO* VAR. *KOI* L.) W BASENACH BETONOWYCH NAWOŻONYCH ORGANICZNIE

Określono wpływ częstotliwości wymiany wody na jej jakość oraz zasobność planktonu i produkcję karpia koi, *Cyprinus carpio* var. *koi* L. w betonowych basenach podchowowych, nawożonych kurzym obor-

nikiem. Porównano indywidualne tempo wzrostu i przeżywalność ryb (masa początkowa 0,09 ± 0,02 g) w czterech wariantach doświadczalnych, w których woda o objętości 100 dm^3 była wymieniana: (1) codziennie (grupa WE1), (2) co drugi dzień (grupa WE2), (3) dwa razy w tygodniu (grupa WE3), (4) raz na tydzień (grupa WE4) - grupa kontrolna (NE) bez wymiany wody. W grupie kontrolnej (NE) stwierdzono istotnie wyższy ($P < 0,05$) poziom następujących wskaźników jakości wody: NH_4-N, NO_2-N, NO_3-N, PO_4-P, konduktywności, zasadowości węglanowej. Zagęszczenie planktonu było najwyższe w grupie WE1 ($P < 0.05$). Przyrost biomasy ryb również był istotnie wyższy w grupie WE1 ($P < 0,05$). Przeżywalność ryb w poszczególnych grupach była istotnie zróżnicowana i wahała się od 60,43% (grupa NE) do 95,21% (grupa WE1). Wyniki badań wskazują, że w podchowie karpia koi w basenach betonowych, nawożonych organicznie, zarówno ze względu na utrzymanie odpowiedniej jakości wody, jak i zagęszczenia planktonu najbardziej efektywna była wymiana 100 dm^3 wody dziennie (grupa WE1).

The Effect of Stocking Density on Growth, Survival Rate, and Number of Marketable Fish Produced of Koi Carps, *Cyprinus carpio* vr. *koi*, in Concrete Tanks

Prithwiraj Jha
Sudip Barat

ABSTRACT. To test the effect of different stocking densities on their growth, survival rate, and number of marketable fish, advanced larvae (0.14±0.035 g) of koi carp, *Cyprinus carpio* vr. *koi*, were cultured for 90 days in 150-L concrete tanks at different densities: 0.1 fish/L (D1); 0.2 fish/L (D2); 0.3 fish/L (D3); 0.4 fish/L (D4); and 0.5 fish/L (D5). There were three replicates for each treatment, where the fish were fed daily, slightly in excess of satiation to eliminate the possibility of food supply being a limiting factor to growth. The D4 and D5 treatments recorded significantly higher ($P < 0.05$) values of ammonium-N, nitrite-N, nitrate-N, phosphate, and specific conductivity, and significantly lower ($P < 0.05$) values of dissolved oxygen, compared to the other treatments. Weight gain for koi carps stocked at D1 (7.28 g) was significantly higher ($P < 0.05$) than that of fish in the other treatments. There was a significant difference in survival rates of koi carps among the treatments ranging from 62.43% in D5 to 93.26% in D1. The number of marketable fish above a set size of 4 g was significantly higher ($P < 0.05$) in D3, compared to other treatments

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with higher or lower stocking densities. Therefore, a stocking rate of 0.3 fish/L would be suggested as optimal.

KEYWORDS. Koi carp culture, *Cyprinus carpio* vr. *koi*, stocking density, growth, marketable fish, water quality

INTRODUCTION

Ornamental fish has grown into one of the world's most popular hobbies. According to a recent report, about 1.5 billion fish worth US$6 billion are traded annually, and the entire industry, including accessories, is estimated to be worth about US $14 billion (Singh and Dey 2003). To supply the growing market, fish farmers need to keep fish at the highest sustainable stocking densities to produce a large number of fish (Olivier and Kaiser 1997). Knowing the optimal stock density is one of the basic factors of intensive fish culture. This density should be the resultant value of the environmental requirements of a given fish species and broadly understood economic efficiency (Holm et al. 1990; Kuipers and Summerfelt 1994; Szkudlarek and Zakes 2002). Fish stocking density is the most sensitive factor determining the productivity of a culture system as it affects growth rate, size variation and mortality (Kaiser et al. 1997).

While the effect of management techniques on growth and production of food fish has undergone intensive investigations, little is known about the growth rates of ornamental fishes under husbandry conditions. In comparison to food-fish production, the densities at which ornamental fishes have been kept are rather low. In Singapore, the largest producer of ornamental fish in Asia, the stocking rate have been reported to be as low as 0.02-0.1 fish/L (Ng et al. 1992) to less then 0.3 fish/L (Fernando and Phang 1985). Among other literatures available, values range from 0.4 fish/L in angelfish, *Pterophyllum scalare* (Degani 1993) and swordtails, *Xiphophorus helleri* (Mondal et al. 2004) to 0.5 fish/L for the gourami, *Trichogaster trichopterus* (Cole et al. 1997). The koi carp, *Cyprinus carpio* vr. *koi* is traditionally cultured at a density of 0.25 fish/L in Hawaii (Asano et al. 2003). However, to our knowledge, there have not been any research studies on stocking rates for koi carp production in tropical condition. The objective of this study was to investigate the effect of different

stocking densities on the growth, survival and number of marketable koi carps. The experiment was conducted in outdoor concrete tanks.

MATERIALS AND METHODS

A total of 675, three-week-old koi carp larvae (0.14±0.035 g), offspring of a mixed commercial production of Kohaku, Asagi, and Bekko koi types, were obtained form a local fish farm (Rainbow Ornamentals, Jalpaiguri, India[1]). After a one-week acclimatization period, fish were randomly assigned to fifteen concrete circular tanks (capacity: 150 L) at densities of 0.1 (D1), 0.2 (D2), 0.3 (D3), 0.4 (D4) and 0.5 fish/L (D5), with three replicates for each density.

All fish were fed three times a day during the 90-day culture period, slightly in excess of satiation to eliminate the possibility that insufficient food supply may influence growth. The fish were given a commercial, floating pelleted diet (Tokyu Company, Japan) containing 32% crude protein, 4% crude fat, 5% crude fiber, 10% crude ash, 9% moisture, and 31% nitrogen-free extract. This level of crude protein corresponds to the established requirement for juvenile cyprinid carps fed to satiation (Lochmann and Phillips 1994) and was selected on the basis of widespread availability. The diet was mechanically crumbled before being administered during the first month; for the rest of the study, original floating pellets (0.24 cm in diameter) was applied. A single layer of plastic bird netting was used to cover the tanks. Since most farmers in India cannot afford aeration facilities, 5% of the water was replaced in each tank every day. The tanks were cleaned daily to remove algae that were attached to the walls.

Water quality parameters (dissolved oxygen, free CO_2, alkalinity, PO_4^{-3}-P, NH_4^+-N, NO_2^--N, NO_3^--N, and specific conductivity) were estimated weekly according to methods as described by APHA (1998). The pH was measured using a portable pH meter (Hanna Instruments, Rua do Pindelo, Vila do Conda, Portugal). Daily temperature was recorded by a centigrade thermometer. The weight of the fish was recorded at the beginning of the experiment, and then, every fortnightly during the culture period. Four random samples of 10 fish from each tank were netted and excess water removed on paper towelling through the net, before the fish were individually weighed. Dead fish were removed daily, and

1. Use of trade or manufacturer's name does not imply endorsement.

were not replaced during the course of study. Differences between the number of fish stocked and the number of fish at harvest were used to calculate percent mortality in each treatment. Fish were harvested after 90 days and weighed (to 1 mg) individually. For this the fish were anaesthetized with tricaine methene sulphonate (MS-222) of 0.04 g/L concentration. The specific growth rate (SGR) was calculated as:

$$SGR = 100\ [(\ln W_t - \ln W_0)/t];$$

where W_0 and W_t are the initial and final live weight of the fish (g), respectively, and (t) is the culture period in days (Ricker 1975). Food conversion rates (FCR) were expressed as diet fed (g) divided by the weight gain (g) of the fish (Olivier and Kaiser 1997). Fish growth, survival, and feed conversion were assessed by one way analysis of variance and Tukey's multiple range test at $P = 0.05$ level of significance (Zar 1996). The number of marketable fish at the end of growth period was calculated using the function for a normal distribution curve, where $z = (y - \mu/\sigma)$; y is the least marketable weight (g), μ is the mean weight of the population, σ is the standard deviation of the total weight, and z follows the standard normal probability distribution which determines the probability of finding fish above a given range. The number of marketable fish (n) was then determined using the table value of the normal probability distribution (P) as follows: n = (I − P) * total number of fish produced.

RESULTS

Fish Growth, Survival, and Feed Conversion

Weight gain of koi carps was affected by the stocking density (Table 1). At harvest, maximum weight gain was achieved in D1, followed in decreasing order by D2, D3, D4, and D5 treatments ($P < 0.05$). All the data related to regressions of the natural log of average fish weight over time for each treatment fitted an exponential model (Table 1). The specific growth rate (SGR) was quite high (> 3.5) in all the treatments, though there were significant differences ($P < 0.05$) within the treatments (Table 1), except D2 and D3, where the SGR did not record significantly differently from each other ($P < 0.05$). Stocking density similarly influenced survival rates of fish, and the D1 (93.36%) and D5 (62.43%) recorded marked significant differences ($P < 0.05$). Calculated FCR values

TABLE 1. Mean±SE harvest weight, weight gain, SGR, and survival rate at the end of three-month growth period (September-November 2002) of koi carps reared in concrete tanks at different stocking densities. Different letters indicate statistically significant differences between means at $P < 0.05$.

	Treatment				
	D1	D2	D3	D4	D5
Harvest weight (g)	7.42±0.05 a	6.69±0.04 b	6.46±0.03 c	4.12±0.07 d	3.48±0.05 e
Weight gain (g)	7.28±0.05 a	6.55±0.04 b	6.32±0.03 c	3.98±0.07 d	3.34±0.05 e
SGR (%/day)	4.38±0.03 a	4.26±0.02 b	4.22±0.02 b	3.72±0.04 c	3.54±0.03 d
Survival rate (%)	93.36±0.89 a	83.40±0.64 b	82.27±0.56 b	73.30±0.28 c	62.43±0.15 d
Feed Conversion Ratio	1.82±0.02 a	2.03±0.02 b	2.07±0.03 b	2.83±0.04 c	2.88±0.03 c
Growth equation[1]	$Y = e^{-1.58 + 0.29\,T}$	$Y = e^{-1.76 + 0.29\,T}$	$Y = e^{-1.85 + 0.29\,T}$	$Y = e^{-2.09 + 0.27\,T}$	$Y = e^{-2.36 + 0.27\,T}$
	$R^2 = 0.84$	$R^2 = 0.89$	$R^2 = 0.91$	$R^2 = 0.96$	$R^2 = 0.98$

[1]The growth models predict mass of fish (Y = g fish) as a function of time (T = weeks).

averaged 1.82-2.88 among the treatments, with the maximum value (2.88) recorded in D5, that had the highest stocking density (Table 1).

Number of Marketable Fish

To determine the output of marketable fish, the percentage and number of fish above a total weight of 4 g was estimated from the probability distribution at the end of the study. Although all the fish collected from the D1, D2, and D3 treatments could be marketed, since they achieved the minimum marketable size, the highest number of marketable fish were produced in D3 (Table 2), which had a stocking density of 0.3 fish/L. When the fish were stocked at densities higher than 0.3 fish/L, it was observed that the number, as well as percentage of fish above the set marketable size (4 g) decreased (Table 2).

Water Quality

Water temperature was between 17°C and 26°C during the 90-day grow-out period. The pH values in all treatments never dropped below 5.8, with a maximum average of 7.05 recorded in the D1 treatment. There were marked differences in water quality among the treatments (Table 3). Values of dissolved oxygen were significantly lower in D4 and D5 ($P < 0.05$), compared to other treatments. Carbonate was absent in all treatments during the entire study period. Average HCO_3^- alkalinity, phosphate, nitrate-N, nitrite-N, and ammonium-N were significantly higher ($P < 0.05$) in D4 and D5, compared to other treatments (Table 3). Specific conductivity was significantly higher in D5, than in other treatments ($P < 0.05$).

DISCUSSION

Growth rate of fishes depend on many factors, but within the limits of the genetic growth potential of the species, they are principally a function of the availability of preferred food. Backiel and LeCren (1967), Hepher (1967), Bardach et al. (1972), Suresh and Lin (1992), Gress et al. (1996), Irwin et al. (1999), Metusalach et al. (1999), and Sharma and Chakrabarti (1999) indicated that growth of many species is density dependent and that there was an inverse relationship between stocking density and individual size of fish produced, primarily because the food supply has to be shared between individuals.

TABLE 2. Average number marketable koi carps (those heavier than 4.0 g) produced, together with marketable fish produced, expressed as a percentage of total number of fish produced (A) and total number of fish stocked (B) in the five treatments with different stocking densities. Different letters in a column indicate statistically significant differences ($P < 0.05$).

Treatment	Stocking density		Number of marketable fish produced (Fish/Tank)	Marketable fish (%)	
	(Fish/L)	(Fish/Tank)		A	B
D1	0.1	15	14 d	100.00	93.36
D2	0.2	30	25 c	100.00	83.40
D3	0.3	45	37 a	100.00	82.27
D4	0.4	60	30.42 b	69.14	50.70
D5	0.5	75	0.05 e	0.11	0.07

TABLE 3. Mean±SE of major water quality parameters analyzed for the five treatments. Each mean represents 14 samples collected at weekly intervals during the 3-month growth period. Different letters in the same row indicate statistically significant differences between means at $P < 0.05$.

Parameters	Treatments				
	D1	D2	D3	D4	D5
pH	7.05±0.04 a	6.93±0.05 a	6.83±0.05 a	6.50±0.08 ab	6.33±0.09 ab
Dissolved oxygen (mg/L)	6.57±0.15 a	6.15±0.18 ab	5.42±0.42 bc	5.24±0.22 c	4.82±0.26 c
Free CO_2 (mg/L)	5.03±0.18 b	5.17±0.20 b	5.53±0.23 b	6.67±0.31 a	6.81±0.31 a
HCO_3^- alkalinity (mg/L)	35.14±0.77 c	37.65±1.16 c	40.22±1.45 bc	47.45±2.33 ab	53.69±3.02 a
NO_3^- - N (mg/L)	0.105±0.006 c	0.151±0.011 bc	0.172±0.014 b	0.235±0.018 a	0.263±0.020 a
NO_2^- - N (mg/L)	0.019±0.001 b	0.021±0.001 b	0.023±0.002 b	0.031±0.003 a	0.034±0.003 a
NH_4^+ - N (mg/L)	0.145±0.005 c	0.175±0.008 bc	0.237±0.016 b	0.357±0.026 a	0.432±0.037 a
PO_4^{-3} - P (mg/L)	0.152±0.011 b	0.182±0.013 b	0.217±0.016 b	0.312±0.024 a	0.342±0.024 a
Specific conductance (mmhos/cm)	0.22±0.002 c	0.23±0.005 c	0.25±0.008 b	0.31±0.013 b	0.37±0.018 a

In our experiment, all the fish were fed to satiation. In addition, in the outdoor tanks, fish had access to algae, hence, food availability alone cannot have caused the differences in the growth rate between the different treatments. It is well known that carp fry respond quite rapidly to different stocking densities in their competition for space, as well as food. Overstocking usually results in low survival rate, while reduced stocking leads to rapid growth of the fry (Rothbard 1982; Rothbard and Yaron 1995). Cage culture experiments in Israel have shown common carp to be extremely sensitive to stocking density (Feldlite and Milstein 1999), where food availability was not a problem, but increased stocking density reduced the space volume available per fish. Violating behavior requirements for space can affect growth through endocrine responses or disruption to feeding efficiency (Pankhurst and Van der Kraak 1997; Schreck et al. 1997). Fox and Flowers (1990) reported on increased losses due to cannibalism in juvenile walleye, *Stizostedion vitreum*, grown at high densities in intensive culture ponds. Cannibalism in many species of fish appears to be directly influenced by the availability of space and shelter (Smith and Reay 1991; Herbert et al. 2003).

Shelton et al. (1981) found that increasing stocking density had a profound negative impact on the growth of grass carp, *Ctenopharyngodon idella*, in small impoundments. Among ornamental fishes, a similar effect of population density on growth rate was found by Olivier and Kaiser (1997) with juvenile swordtails, *Xiphophorus helleri*, fed to satiation, and Degani (1993) with angelfish, *Pterophyllum scalare*, although, the latter experiment was conducted indoors and the initial size of fish were more than 1g. For our experiment, advanced larvae (0.14±0.035 g) of koi were selected, since, tropical fish breeders in India usually sell fish larvae (of 2-3 weeks age) to fish growers.

Large-scale fish production involves fry stocking in earthen ponds from where marketable fish are harvested after 3-4 months of culture. One of the bottlenecks in this industry is the large-scale loss of fish during the post-larval stage, where mortality rates are very high before the fish reach 1g size. Given the sensitivity the koi carps have shown to increasing stocking density in the present experiment, more studies on husbandry aspects of koi carp rearing needs to be conducted that would enable fish produces to increase survival rates, thereby increasing profits without further investments.

Environmental conditions in the culture tanks were influenced by the stocking density. High accumulation of excrements and metabolic wastes from the fish led to significantly higher concentrations of nitrogen

compounds and simultaneously lowered the dissolved oxygen in D4 and D5, compared to other treatments. Low dissolved oxygen is considered as one of the limiting factors to fish production. A clear-cut relationship between fish yields and increasing levels of aeration was documented for *Tilapia* sp. (Teichert-Coddington and Green 1993). Repeated exposure to low dissolved oxygen can slow the growth process in goldfish, *Carassius auratus* (Stone et al. 2003).

Different species are differently sensitive to nitrogen toxicants. One of the common problems in koi carp culture is depression of feeding when water quality is sub-standard. Asano et al. (2003) observed periods of depressed feeding in koi carps cultured in tanks when ammonia levels were high and dissolved oxygen was low. Although unionized ammonia was not estimated in our experiment, it is known to be in equilibrium with the development of ammonium ions in water (Barat and Jana 1990).

Nitrite is also toxic to many species of fishes (Barat and Jana 1991). Korwin-Kossakowski and Ostaszewska (2003) reported on adverse impact on the growth of common carp due to nitrite exposure. Compared to other nitrogen toxicants, nitrate is toxic only at very high levels (Asano et al. 2003). Although daily water exchange helped in controlling the ammonium, nitrite and nitrate concentration in the tanks, the significantly higher growth and survival rates of koi carps in the D1, D2 and D3 treatments, compared to D4 and D5, could be influenced by the better water quality in terms of higher dissolved oxygen and lower levels of nitrogen toxicants. Jana and Barat (1992) reported marked changes in water quality due to high stocking load of fish in culture tanks. Physiological stress and impaired growth due to poor water quality associated with crowding have been reported in rainbow trout, *Oncorhynchus mykiss* (Zoccarato et al. 1994) and summer flounder, *Paralichthys dentatus* (King et al. 2000).

The results from the probability distribution table (Table 2) indicate that stocking koi carps at a density of 0.3 fish/L, rather than at higher or lower densities, yielded the highest number of marketable fish. In contrast to food fish production, where producers focus primarily on the total number of fish produced (Jolly and Clonts 1993), ornamental fish can only be sold when they have reached a particular size. While the results consistently showed that increasing density reduced growth (Table 1), it would appear logical to reduce the stocking density, so that a faster growth rate is allowed, and the fish can quickly reach the smallest marketable size (4 g).

However, no significant increase in price for koi carps that grow larger than the minimum marketable size (4 g) in India provide strong financial incentives for farmers to maintain fishes at the smallest marketable size.

The goal of production is to produce the highest number of fish of the given size (4 g) with consistently low size variation. Hence, although the weight gain of koi carps was considerably higher in D1 and D2 treatments (Table 1), D3 (0.3 fish/L) seemed to be the optimal density for stocking koi carps, since, the number of marketable fish was highest in that treatment.

As an adjunct to maintaining density, farmers could seek to increase or reduce fish growth through genetic selection. The fry of the European race of common carp cultured under crowded conditions were found to grow several times more slowly than the fry of the Chinese race (Hualata et al. 1982). It should be remembered that the term 'koi' describes many strains of ornamental carp that have been genetically selected over many generations (Feldlite and Milstein 1999). Thus, one should not be surprised to find results with different growth and survival rates to those reported here for the offspring of mixed Kohaku, Asagi, and Bekko koi types.

The results suggest average size and survival rate of koi carps to be inversely related to stocking density, and, are in agreement with earlier studies with other fish species (Shelton et al. 1981; Degani 1993; Stone et al. 2003). From the data obtained, it can be recommended to stock koi carp fry at a density of 0.3 fish/L. The productivity should be measured in terms of number of marketable fish, which would favor a density of 0.3 fish/L, compared to higher or lower stocking densities.

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MANAGEMENT INDUCED CHANGES IN FOOD SELECTION, GROWTH AND SURVIVAL OF KOI CARP, *CYPRINUS CARPIO* VAR. *KOI* L., IN TROPICAL PONDS

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Abstract

The effects of different management regimes on the feeding habits and food selection of koi carp (*Cyprinus carpio* var. *koi* L.) larvae were examined. Weight gain, fish deformities, and survival were compared in an 11-week growth trial conducted in tropical ponds maintained according to four culture regimes: (1) live food system; (2) poultry manure treated system; (3) cow dung treated system; and (4) a control. The Ivlev's Electivity Index showed that koi larvae avoided phytoplankton and preferred cladocerans, an important source of natural food in all the regimes. In the poultry and cow manured ponds, the larvae were negatively elective towards copepods although they were more abundant than cladocerans. Weight gain and survival was significantly higher in the live-feed system ($p<0.05$) than in the other systems. Fish deformities were significantly higher ($p<0.05$) in the control.

Introduction

One of the bottlenecks in the ornamental fish culture industry in India is the large-scale loss of fish during the larvae and postlarvae periods. To explain differing survival rates, early marine studies focused on the feeding behavior of young larvae (Hjort, 1914). Even in modern aquaculture, food is considered the most powerful variable affecting growth and metabolism (Kinne, 1962; Beamish and Dickie, 1967; Miller et al., 1988; Bunnell et al., 2003). Natural food is indispensable in the early life of fish (Crowder et al., 1987; Hart and Werner,

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1987; Rottmann et al., 1991; Adeyemo et al., 1994; Welker et al., 1994; Al-Harbi and Siddiqui, 2001). Management strategies of fish husbandry are likely to influence species composition and abundance of plankton in the environment (Diana et al., 1991; Milstein et al., 1995; Garg and Bhatnagar, 1996; Akpan and Okafor, 1997; Jakubas, 2002; Mischke and Zimba, 2004) which could, in turn, affect the feeding habits, growth, and survival of the target fish species.

Two types of plankton feeding behavior are particulate feeding and filter feeding. In nature, switching from particulate to filter feeding behavior is a function of factors such as the density and size of the prey (Lazzaro, 1987; Dewan et al., 1991; Ushakumari and Aravindan, 1992; Xie, 1999; Serajuddin, 2000). Some plankton pass undigested through the gut of planktivorous fishes. In this case, since the fish expends energy to capture prey but receives no energy from consuming it, the fish may recognize and reject such undesirable organisms. Ivlev (1961) and Vinyard (1967), respectively, reported on slightly negative electivity towards ostracods by bleak, *Alburnus alburnus* L., and bluegill, *Lepomis macrochirus* Raf.

The koi carp, *Cyprinus carpio* var. *koi* L., is a popular ornamental fish. There are several reports on the husbandry of this fish (Feldlite and Milstein, 1999; Asano et al., 2003). However, to our knowledge, there have been no studies of the feeding habits and food preferences of the koi in Indian conditions. The objective of the present study was to investigate the food selection, growth, and survival of koi larvae reared under different management regimes in tropical ponds.

Materials and Methods

Study animals. Koi carp (*Cyprinus carpio* var. *koi* L.) larvae (0.13±0.015 g) from mixed commercial production by 40 pairs of *asagi*, *bekko*, and *showa* koi were obtained from a local fish farm (Rainbow Ornamentals, Jalpaiguri, India), and divided into two batches after a one-week acclimatization period.

Experimental design. The first batch was reared in 12 earthen ponds for 11 weeks (March 3-May 19, 2004) and used for the growth and survival studies. Each pond (9.14 x 6.10 x 1.07 m) had a capacity of 59,600 l. Larvae were stocked at the optimum density of 0.3 fish/l (Jha and Barat, in press). Three ponds were allotted to each of four management regimes: (a) live food ponds, into which about 1000 l of zooplankton water was transferred every day from plankton culture ponds. The plankton ponds were fertilized with 0.26 kg poultry manure/m^3 at the beginning of culture and every 10 days thereafter (Jha et al., in press); (b) poultry manure ponds, where poultry excreta was added to the koi larvae ponds at the same dose as above; (c) cow dung ponds, where cow dung was applied to the koi ponds at the above dose; and (d) control ponds, in which a commercial pelleted feed (Tokyu Corp., Japan) containing 32% crude protein was used.

Constant water levels were maintained in the culture ponds by supplying ground water to compensate for loss due to evaporation. In the live food treatment, about 1000 l of water was discharged every day during the introduction of the plankton water. A plankton cloth was tied over the outflow water pipe to prevent any loss of zooplankton during this process.

The second batch was used to study food selection. A 10-cm layer of soil was placed on the bottom of six 2000-l outdoor concrete tanks exposed to direct sunlight which were then filled with control pond water. The tanks were fertilized with 0.26 kg poultry manure/m^3 two weeks prior to stocking the carp fry and once every 10 days thereafter (Jha et al., in press). The koi were stocked and maintained at a density of 0.3 fish/l. Forty-eight fish were randomly removed at weekly intervals. Each fry was placed in a plastic container (5 l capacity) and starved for 48 h for gastric evacuation under laboratory conditions. Four containers, each containing one fry, were randomly placed on the bottom of different areas of each of the above twelve ponds (three for each management regime). The containers were covered with a net to prevent fish escape but allow free movement of the plankton between the container and the surround-

ing pond water. Hence, the containers represented the management regime of the pond in which they were placed in terms of water quality and plankton diversity. After 12 h, the fish were removed from the containers and sacrificed for examination of food selection and food consumption.

Data collection. Consumed plankton were identified and counted by analyzing the guts of koi in the second batch. Routine procedures were followed for gut analysis (Jhingran et al., 1988). Average values from the 12 containers used for each regime were used for further calculations. The extent of prey selection was determined using the formula of Ivlev (1961): $E= (r_1 - p_1)/(r_1 + p_1)$, where E is the electivity value, r_1 is the relative quantity of any ingredient in the gut expressed as a percent, and p_1 is the relative quantity of the same ingredient in the food complex, also expressed as a percent. Application of this formula results in a range of values from +1.0 indicating a very high degree of selection to -1.0 for complete avoidance. A value of 0 indicates that the feed is present in the diet in the same proportion as it is found in the environment, viz. a complete lack of selection.

Water samples were collected from the ponds at weekly intervals and analyzed according to methods described by APHA (1998). pH was measured *in situ* using a portable pH meter (Hanna Instruments, Rua do Pindelo, Portugal). Temperature was measured with a centigrade thermometer. Samples of plankton were collected with a plankton net made of standard bolting silk cloth (no. 21 with 77 mesh/cm^2) twice a week. Plankton samples were concentrated to 20 ml and preserved in 4% formalin. Plankton in a 1-ml concentration were counted under a stereoscopic microscope using the Sedgwick Rafter Counting Cell.

For growth studies, 1000 fish were randomly collected from each pond and individually weighed to the nearest 0.001 g at the beginning of the experiment and at harvest and the number and percent of fish with deformities were recorded. Dead fish were removed daily, they were not replaced during the course of study, and differences between the number of fish stocked and the number of fish at harvest were used to calculate survival. Final survival and percent of deformities were normalized using angular transformation (Sokal and Rohlf, 1969). The specific growth rate (%/day) for each treatment was calculated using the formula of Ricker (1975).

Statistical analysis. Analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference Test were used to determine significant differences between groups with respect to water quality, fish growth and survival, and number of deformed individuals.

Results

The compositions of planktonic food organisms in the environment and gut contents are presented in Table 1. Cladocerans were in higher abundance in the diet (82.14%) and environment (63.89%) than copepods (17.85% and 29.69%, respectively) in the live food treatment. Cladocerans were higher in the live food treatment than in the other treatments. In the cow dung treatment, cladocerans were more abundant in the fish gut (44.71%) than copepods (40.12%). *Moina* was the most dominant cladoceran in all the treatments, ranging in the environment from 5% in the control to 27.95% in the live food treatment and in the gut from 6.34% in the control to 31.81% in the live food treatment.

Copepods were more abundant in the control and manured ponds, ranging from 48.71% in the cow dung treatment to 61.33% in the control. *Cyclops* was the most dominant copepod in all the treatments. The average number of plankton per liter was highest in the live food treatment, followed by the poultry manure, cow dung, and control treatments, in that order ($p<0.05$; Fig. 1).

The larvae preferred cladocerans in all the treatments and were generally negative towards copepods except in the control where the electivity index was insignificant (0.005) and indicated an absence of any food selection. Electivity of rotifers was negative in all treatments with values ranging from -0.181 in the poultry manure treatment to -1.0 in the live food treatment. Likewise for phytoplankton

Table 1. Percent plankton in the gut of koi carp larvae and in experimental ponds, and Ivlev's Electivity Index*.

	Live food			*Poultry manure*			*Cow dung*			*Control*		
	% in gut	*% in environment*	*Ivlev Index*	*% in gut*	*% in environment*	*Ivlev Index*	*% in gut*	*% in environment*	*Ivlev Index*	*% in gut*	*% in environment*	*Ivlev Index*
Daphnia	22.25	14.72	0.204	12.72	7.36	0.267	14.08	7.31	0.316	4.94	3.06	0.235
Moina	31.81	27.95	0.065	18.13	11.69	0.216	18.24	11.93	0.209	6.34	5.00	0.118
Ceriodaphnia	25.26	19.61	0.126	10.15	7.01	0.183	10.52	7.75	0.152	4.28	3.62	0.084
Bosmina	2.91	1.59	0.293	1.28	1.32	-0.015	1.86	1.89	-0.008	4.51	3.03	0.196
Cladocera	**82.14**	**63.89**	**0.125**	**42.29**	**27.41**	**0.213**	**44.71**	**28.90**	**0.215**	**20.08**	**14.73**	**0.154**
Cyclops	11.89	16.67	-0.167	21.62	24.84	-0.069	18.78	23.49	-0.111	32.26	31.80	0.007
Diaptomus	-	0.90	-1.0	6.90	7.63	-0.050	4.19	6.48	-0.215	11.56	11.49	0.003
Nauplii	5.96	12.11	-0.340	17.59	18.84	-0.034	17.14	18.72	-0.044	18.19	17.93	0.007
Copepoda	**17.85**	**29.69**	**-0.249**	**46.12**	**51.32**	**-0.053**	**40.12**	**48.71**	**-0.097**	**62.02**	**61.33**	**0.005**
Brachionus	-	1.60	-1.0	3.51	4.87	-0.162	2.29	4.69	-0.344	3.92	5.04	-0.125
Keratella	-	0.67	-1.0	2.63	3.97	-0.203	0.87	3.75	-0.623	2.19	4.16	-0.310
Rotifera	**-**	**2.29**	**-1.0**	**6.14**	**8.85**	**-0.181**	**3.16**	**8.44**	**-0.455**	**6.12**	**9.21**	**-0.201**
Chlorella	-	0.81	-1.0	0.59	2.25	-0.585	1.82	2.63	-0.182	2.51	3.76	-0.199
Navicula	-	1.91	-1.0	2.32	4.75	-0.344	4.92	5.47	-0.053	4.28	5.14	-0.091
Spirogyra	-	0.63	-1.0	1.02	1.76	-0.266	1.60	1.71	-0.033	2.05	2.65	-0.128
Scenedesmus	-	0.04	-1.0	-	0.25	-1.0	-	0.46	-1.0	-	0.30	-1.0
Phacus	-	0.58	-1.0	1.50	3.03	-0.338	3.65	3.18	0.069	2.93	2.03	0.181
Synedra	-	0.12	-1.0	-	0.33	-1.0	-	0.46	-1.0	-	0.82	-1.0
Phytoplankton	**-**	**4.12**	**-1.0**	**5.44**	**12.42**	**-0.391**	**12.00**	**13.94**	**-0.075**	**11.77**	**14.73**	**-0.112**

* Electivity index = $(r_1 - p_1)/(r_1 + p_1)$, where r_1 is the relative quantity of the ingredient in the gut expressed as a percent, and p_1 is the relative quantity of the same ingredient in the food complex, also expressed as a percent.

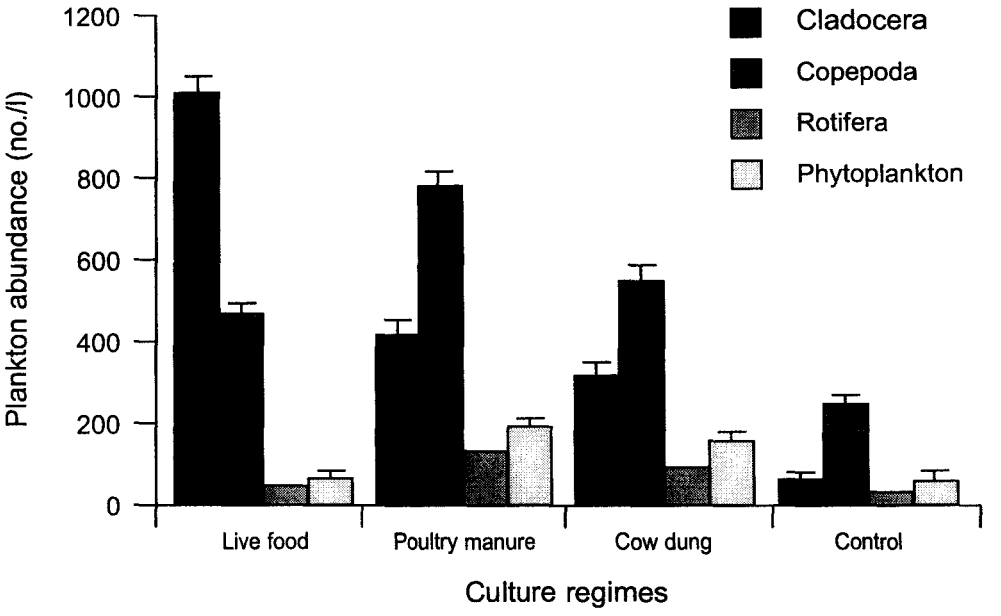

Fig. 1. Average number of plankton per liter.

which ranged from -0.075 in the cow dung ponds to -1.0 in the live food ponds.

Water temperature ranged 27-36°C with no differences between treatments at any time. pH and dissolved oxygen were significantly higher in the live food treatment than in the manured treatments (Table 2). Average specific conductivity and nitrite-N were significantly higher in the poultry ponds than in the others. Total alkalinity, here referring to bicarbonate alkalinity as carbonate, was absent in all treatments. Alkalinity, BOD, phosphate-P, ammonium-N, and nitrate-N were significantly higher in the manured treatments than in the live food and control treatments.

At harvest, the highest weight gain and specific growth rate were obtained in the live food treatment (Table 3). The number of deformed carp was highest in the control. Survival significantly differed among treatments, ranging 70.60-96.16%.

Discussion

Electivity indices ranging from -0.3 to +0.3 are generally considered insignificantly different from zero, and thus indicate non-selective feeding (Lazzaro, 1987). According to this interpretation, the koi larvae in our experiment did not show any significant food selectivity towards most planktonic organisms. Analyzed by plankton types, a strong rejection (below -0.3) was observed only towards phytoplankton in the live food and poultry manure treatments and towards rotifers in the live food and cow dung treatments. There were no incidences of strong selection (above +0.3). Analyzed by individual plankton, there was only one incidence of strong positive selection (towards *Daphnia* in the cow dung treatment).

Xie and Takamura (1996) and Serajuddin (2000), however, defined electivity values above +0.01 as positive and below -0.01 as negative, reducing the non-selective feeding

Table 2. Water quality parameters (means of 12 samples collected weekly for 11 weeks±SE).

	Treatment			
Parameter	*Live food*	*Poultry manure*	*Cow dung*	*Control*
pH	7.42±0.11 a	6.67±0.13 b	6.03±0.15 c	7.19±0.09 ab
Dissolved oxygen (mg/l)	7.32±0.10 a	5.19±0.23 b	5.67±0.18 b	6.97±0.13 a
BOD (mg/l)	1.67±0.06 c	2.61± 0.14 a	2.18±0.10 ab	1.82±0.08 bc
Free CO_2 (mg/l)	2.57±0.10 c	3.47±0.12 a	3.18±0.16 ab	2.84±0.13 bc
Total alkalinity (mg/l)	31.75±1.18 b	79.92±5.14 a	70.25±4.28 a	34.42±1.86 b
PO_4-P (mg/l)	0.23±0.021 b	0.53±0.059 a	0.45±0.039 a	0.28±0.024 b
NH_4-N (mg/l)	0.151±0.014 b	0.332±0.032 a	0.273±0.024 a	0.295±0.027 a
NO_2-N (mg/l)	0.009±0.001 c	0.034±0.003 a	0.021±0.002 b	0.012±0.001 c
NO_3-N (mg/l)	0.164±0.014 b	0.412±0.045 a	0.343±0.032 a	0.19±0.016 b
Specific conductivity (mmhos/cm)	0.26±0.016 c	0.64±0.041 a	0.46±0.026 b	0.27±0.012 c

Different superscripts in a row indicate statistically significant differences ($p<0.05$).

Table 3. Growth (means±SE), rate of deformities, and survival in koi raised in different management regimes.

	Treatment			
Parameter	*Live food*	*Poultry manure*	*Cow dung*	*Control*
Harvest weight (g)	8.67±0.16 a	6.23 ± 0.18 b	4.37 ± 0.12 c	3.56 ± 0.11 d
Weight gain (g)	8.54±0.16 a	6.10±0.18 b	4.24±0.12 c	3.43±0.11 d
SGR (%/day)	5.45±0.14 a	5.03±0.10 b	4.56±0.07 c	4.30±0.07 d
Deformed individuals (%)	1.9 d	10.05 b	5.57 c	18.07 a
Survival rate (%)	96.16 a	90.5 b	81.86 c	70.60 d

Different superscripts in a row indicate statistically significant differences between means ($p<0.05$).

range to -0.01 to +0.01. According to this definition, food selectivity of koi larvae was clearly demonstrated in our results, with positive selection of cladocerans and negative selection of other groups.

Cladocerans were found in larger proportions in the diet than in the environment in all the treatments, implying that cladocerans constitute an important source of natural food for koi larvae in any culture system. The positive selection of cladocerans in all the treatments suggests that koi larvae prefer cladocerans despite the dominance of copepods in all but the live food treatment. This shows that koi larvae do not necessarily feed on the most abundant type of plankton. In the live food treatment, the cladoceran dominance resulted from the introduction of supplemental zooplankton cultured in plankton culture ponds with cladocerans as the major inoculum.

The feeding strategy of planktivores is based on the structure and functioning of their branchial feeding apparatus viz. gill rakers (Serajuddin, 2000). The presence of mucous helps to consolidate and transport food, possibly improving the retention efficiency of the filter. Characteristics such as the shape and size of the suspended particles and alteration capabilities of the mesh size of gill rakers also play important roles in food retention (Serajuddin, 2000). Food items may be rejected because they are larger than the mouth size of the fish.The koi larvae in our experiment were relatively young (0.13-8.67 g), and their mouth size may have prevented their consuming larger plankton. In nature, the small size of the carp fry mouth (Dabrowski and Bardega, 1984) acts as a constraint for optimal diet breadth during early stages (Werner, 1974).

The avoidance of food organisms may also be linked to taste, especially when fish probe the aggregation of food items and as demonstrated by the differential secretion of mucous by grass carp *(Ctenopharyngodon idella* Val.) in varied food conditions (Omarova and Lazareva, 1974). Negative selectivity in the manured and control treatments to outright rejection in the live food treatment of phytoplankton agrees with earlier experiments with other fish species including *Catla catla* Ham. (Jafri and Mustafa, 1975), brown trout, *Salmo trutta* L. (Fitzmaurice, 1979), and common carp, *Cyprinus carpio* L. (Chakrabarti and Jana, 1990).

The higher weight gain, SGR, and survival in the live food treatment could be attributed to the significantly higher abundance of cladocerans in that treatment. The highest concentration of zooplankton was in the live food treatment because of the regular addition of plankton to the ponds and as a result of the improved water quality (lower BOD, ammonium, and nitrite; higher DO and pH) that is conducive to reproduction of some of the zooplanktons that constitute the main food items for carp (Jana and Chakrabarti, 1993). The plankton intake of planktivorous fishes varies with feeding conditions. Jana and Chakrabarti (1990) reported that plankton intake of common carp, *Cyprinus carpio* L., in a live food system was higher than in a manured or control system.

The direct relationship between plankton intake and average body weight was demonstrated in carp by Chakrabarti and Jana (1991). The significantly lower weight gain, SGR, and survival rate in the control may have been due to an insufficient quantity of plankton in the system. From the experimental results, it seems that the larvae did not prefer the imported pelleted feed provided in this treatment, similar to results obtained in an earlier experiment (Jha et al., in press).

The observed deformities were mostly scoliosis and bent fins. Ornamental fish, unlike food fish, must be visually attractive to be marketable; deformed fish are aesthetically unattractive to potential customers. The percentage of deformed fish in the various treatments cannot be explained by the available data. The absence of any earlier report relating deformities in koi to husbandry management makes it difficult to draw conclusions. The deformities may have a genetic background since the experimental larvae were the offspring of a mixed commercial production of different koi types and randomly stocked in the ponds. They may also have been environmentally induced. The significantly higher percent of deformed fish in the

control could be attributed to the lower abundance of plankton in the environment. A high incidence of deformities in fish fed commercial food was reported for crucian carp, *Carassius carassius* L. (Myszkowski et al., 2002).

From the findings of the present investigation, in which food selection of koi carp larvae reared under different management regimes in tropical ponds was reported for the first time, raising koi carp larvae in live food ponds with added plankton appears to be a better alternative than the conventional system of applying poultry manure or cow dung.

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BEHAVIOURAL RESPONSES OF TWO POPULAR ORNAMENTAL CARPS, *CYPRINUS CARPIO* L. AND *CARASSIUS AURATUS* (L.), TO MONOCULTURE AND POLYCULTURE CONDITIONS IN AQUARIA

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Abstract. Experiments were conducted to document behavioural responses of koi carp, *Cyprinus carpio* L., and goldfish, *Carassius auratus* (L.) to monoculture and polyculture conditions in aquaria. Two parallel experiments, otherwise involving similar experimental protocols, were carried out with two batches of fish, fed with live tubifex worm (first batch) and live zooplankton (second batch). Each of the trials, randomized with respect to treatment, yielded data on aggressive encounters (chases, nips), both in presence and absence of food. The two species exhibited considerable variation in the extent and type of aggression displayed, koi carp being the more aggressive species. Frequency of attack increased in the presence of food. The impact of aggressive behaviour of koi carp was conspicuous by the increased level of attack on goldfish in polyculture trials in both experimental batches.

Key words: koi carp *Cyprinus carpio*, goldfish, *Carassius auratus*, aggressive behaviour, monoculture, polyculture conditions

INTRODUCTION

Fish polyculture is attempted by stocking two or more species with different feeding habits and different habitat preferences (Lutz 2003). The productivity of the aquatic system is thus increased by introducing more than one compatible fish species in a culture system, where the growth and survival of one species is not negatively affected by the other. To test the interactions between species in polyculture systems, behavioural studies on fish-fish interactions are suitable evaluation tools (Milstein 1992, Kramer et al. 1997, Shumway 1999).

Prominent among freshwater ornamental carps cultured in India are koi carp, *Cyprinus carpio* L., and goldfish, *Carassius auratus* (L.). Since the marketable size of koi carp and goldfish are quite similar (> 4.0 g), and both species require a similar culture period of 11 to 12 weeks to attain the marketable size (Jha and Barat 2005 a), there is a general tendency among fish producers and hobbyists to stock them together. However, further work on the impacts of polyculture on the overall culture performance of each species is necessary. Behavioural studies on the interspecific interrelationships is one tool for evaluation. The objective of the present study was to compare the behavioural responses of koi carp and goldfish stocked in mono- and polyculture combinations to assess their behavioural compatibility.

MATERIALS AND METHODS

About 2- to 3-week-old larvae of bekko type koi carp, *Cyprinus carpio* L. (0.13 ± 0.03 g; $n = 50$), and goldfish, *Carassius auratus* (L.) (0.18 ± 0.05 g; $n = 50$) were collected from a local hatchery (Rainbow Ornamentals, Raninagar, Jalpaiguri, India) and transferred to a laboratory at the University of North Bengal, where they were acclimated for nearly three weeks prior to the study. At first, the koi carp and goldfish larvae were divided into two batches. Both batches were subjected to similar acclimation and experimental protocols, except for the food applied to each batch, which was different. The fish were maintained in aerated and filtered aquaria, under artificial lights (12 L : 12 D photoperiod). The tanks were filled with tap water (water volume 150 L; 20 fish/tank). The fish were fed live tubifex worm (first batch) and live zooplankton (second batch) during this period, offered daily in the amount of 5% body weight of the fish stocked (the actual amount of food was adjusted every weekly). The same food was used later during the experiments. The tubifex food was procured from the local market, while zooplankton was cultured in con-

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crete tanks under protocols described earlier (Jha and Barat 2005 b). The physico-chemical conditions were similar in all the holding tanks (temperature 24–26°C; pH 7.0–7.2; dissolved oxygen content 6.5-6.8 mg · L^{-1}).

Two parallel experiments were conducted for the two batches during 15 consecutive days from 20 August to 3 September, 2004 in six 150-L tanks (three tanks for each experiment) maintained under physico-chemical conditions similar to those of the holding tanks (temperature 23–26°C; pH 6.9–7.2; dissolved oxygen 6.5–6.9 mg · L^{-1}). In these trials, monoculture or polyculture groups totalling 20 fish were used in each aquarium. The treatments (for each batch) were: (1) koi carp, monoculture; (2) goldfish, monoculture; and (3) koi carp and goldfish, polyculture, stocked at 1:1. On the average, the 5- to 6-week-old fish used during the study had a mean weight of 0.86 ± 0.11 g (koi carp; $n = 50$) and 1.49 ± 0.38 g (goldfish; $n = 50$).

The order of trials was randomized with respect to treatment and 15 replicate trials (one trial every day) were performed for each treatment. Grid markings on the tanks allowed the location (depth) of the fish in the water column to be estimated. The tanks were screened with black art paper from four sides and an eye slit was cut on the front side, which allowed the experimenter to observe and record the behaviour of fish without disturbing them. In each trial, the fish were placed in the tank and left to settle for 1 h. This was followed by a period of focal sampling (Martin and Bateson 1990) for 5 minutes in which the occurrence and direction of all antagonistic interactions (chases, nips) involving a randomly chosen individual of each species were recorded. Then, a small amount of food was applied and the above procedures were repeated. Each trial therefore yielded data on aggressive encounters of the species concerned, both before food application and in the presence of food.

Aggressive behaviour was measured as the number of attacks, defined as accelerated swimming movements or a sudden burst of speed by one fish towards another (chases), followed by nips—a bite, and/or flight or pursuit. In the first batch, live tubifex were applied in feeding baskets and placed on the floor of the tanks. For the second batch, the food (plankton) was added centrally onto the water surface and the live plankton dispersed rapidly over the entire water surface. This way of offering the food generally increases feeding opportunity and promotes uniformity of feeding and growth (McCarthy et al. 1999, Gomez-Laplaza 2002).

Differences in the mean chasing and mean nipping frequency recorded for each species between the monoculture and polyculture treatments were examined using Student's paired t-test (Student 1908, Gupta 2000). Significance was accepted at $P < 0.05$. Separate analyses were carried out on the "food present" and "food absent" (before application of food) data for each batch. Primary data (number of nips and chases) were log-transformed prior to the analysis and standardised by dividing the number of attacks received by focal fish per trial by the number of potential aggressors. For interspecific attacks, the number of aggressors was n, where n = group size of the attacking species. For conspecific attacks, the number of aggressors was $n - 1$.

RESULTS

Attack levels as estimated from the standardised chasing and nipping frequency per trial for both species were significantly higher in polyculture treatments, compared to monoculture treatments, both before food application and in the presence of food ($P < 0.05$). The attack level also increased markedly in the presence of food, compared to when food was absent in both experimental batches (Table 1). These aggressive acts were very short, and often did not involve actual physical contact. When physical contact occurred, in most cases it involved a momentary pinch by pushing the snout of one fish against the abdomen or fins of another. Total attacks per trial (chases plus nips) as estimated from the different species level interactions (Table 1) showed that goldfish received more attacks under polyculture conditions in both batches, compared to koi carp. Under monoculture conditions, the incidence of attacks in goldfish tanks were lower compared to koi carp tanks, marking the koi carp as a more aggressive species. Chasing (Figs. 1 and 2) and nipping (Figs. 3 and 4) frequencies followed similar trends, although the chasing frequency was higher than the nipping frequency (Table 1).

DISCUSSION

The two species exhibited considerable variation in the extent and type of aggression displayed. Goldfish in monoculture treatments appeared less aggressive, compared to monocultured koi carp in both experimental batches. Even in the polyculture treatments, goldfish attacked conspecifics or other species (koi carp) very rarely. On the other hand, koi carp were overwhelmingly more aggressive. The frequency of attack increased significantly in the presence of food. Food was shown to increase the rates of aggression in gouramis, *Trichogaster trichopterus* (Pallas) (Syarifuddin and Kramer 1996) and poeciliids, *Gambusia holbrooki* (Girard) and *Xiphophorus helleri* Heckel (Warburton and Madden 2003). The broader diversity of species-specific behaviours and salient stimuli may also have enhanced the levels of activity. In a study of conspecific and interspecific interactions between brook trout, *Salmo gairdneri* (*Oncorhynchus mykiss* (Walbaum)), Newman (1956) postulated that the presence of food increased feeding activity, which in turn increased aggressive activity as the focus of attacks was displaced from the food to fellow fish of both species. He further noted that feeding fish displayed some movements that are associated with aggression, such as body undulations, swift darting and biting, and suggested that such movements constituted sign stimuli eliciting attacks from other species.

The significantly higher rate of attacks in the polyculture treatments compared to monoculture conditions for both batches of fish undermines the very logic behind stocking koi carp and goldfish together. Although the impact of nipping on the rate of spinal and caudal abnormalities or fin deformities were not estimated in the

Table 1

Standardised frequency of attacks calculated for koi carp (K) and goldfish (G) in the two experimental batches

Interaction	*n*	Total attacks/trial	Nips : chases	Food : no food
		First Batch (Live tubifex as food)		
Monoculture				
K → K	982	1.258	0.426	2.778
G → G	496	0.544	0.424	3.945
Polyculture				
K → K	345	0.913	0.520	3.492
K → G	2753	2.944	0.584	1.769
G → G	328	0.846	0.065	1.735
G → K	161	0.077	0.185	+
		Second Batch (Zooplankton as food)		
Monoculture				
K → K	660	0.845	0.335	1.807
G → G	369	0.294	0.556	2.379
Polyculture				
K → K	233	0.571	0.903	1.584
K → G	2246	2.708	0.605	1.396
G → G	537	1.330	0.360	1.509
G → K	174	0.156	0.902	+

"Total attacks" = chases plus nips; (+) indicates an increase in the presence of food, but where a ratio cannot be calculated due to a zero "no food" value

present experiment, it could be suggested that sustained attacks, particularly on goldfish by koi carp under polyculture conditions could induce stress and increase the rate of deformities in a pond polyculture. It should be remembered that ornamental fish need to be visually attractive to be acceptable on the market, and deformed or stressed fish could be aesthetically unattractive to potential customers (Jha and Barat 2005 b).

The two food items used the two experimental batches (tubifex and plankton) were selected with a due consideration to the food availability under pond conditions. The impact of aggressive behaviour of koi carp was clearly demonstrated by the increased level of attack on goldfish in the polyculture treatments in both the experimental batches. Working with introduced poeciliid, *Gambusia holbrooki* and native Australian fish, *Pseudomugil signifer* Kner, Howe et

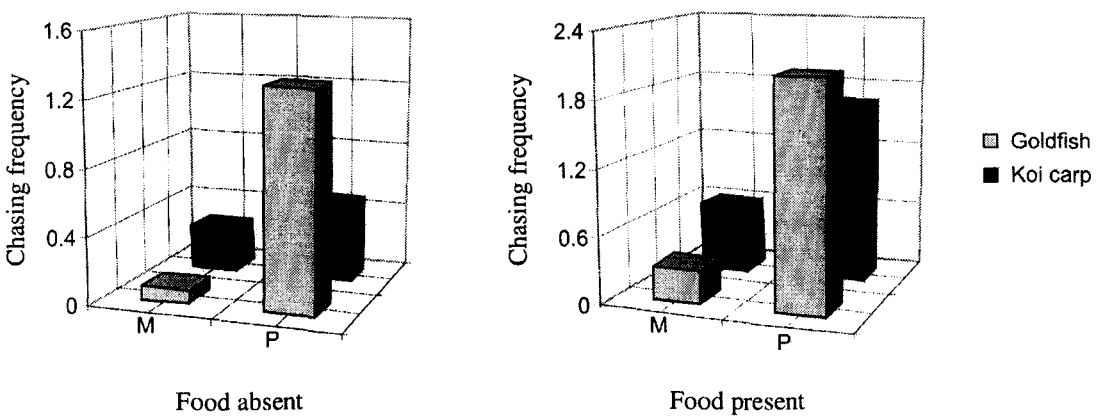

Fig. 1. Standardised (per capita) chasing frequency per trial estimated for koi carp and goldfish under monoculture (M) and polyculture (P) conditions in aquaria in the presence and absence of food (tubifex)

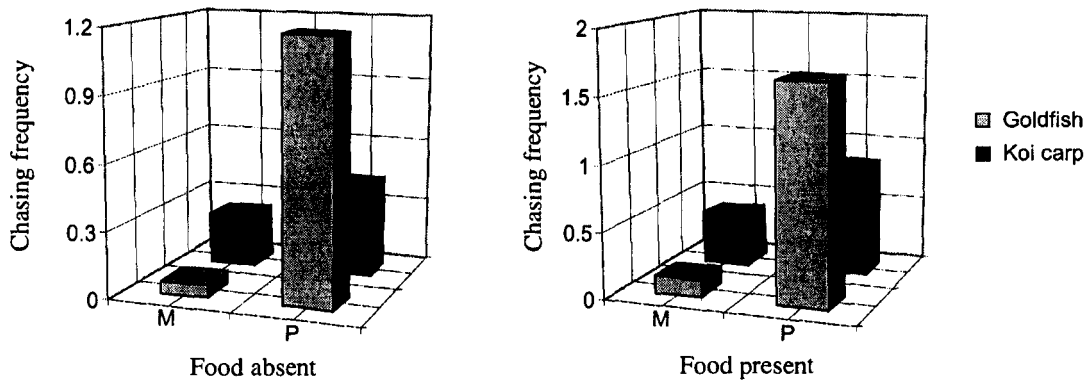

Fig. 2. Standardised (per capita) chasing frequency per trial estimated for koi carp and goldfish under monoculture (M) and polyculture (P) conditions in aquaria in the presence and absence of food (plankton)

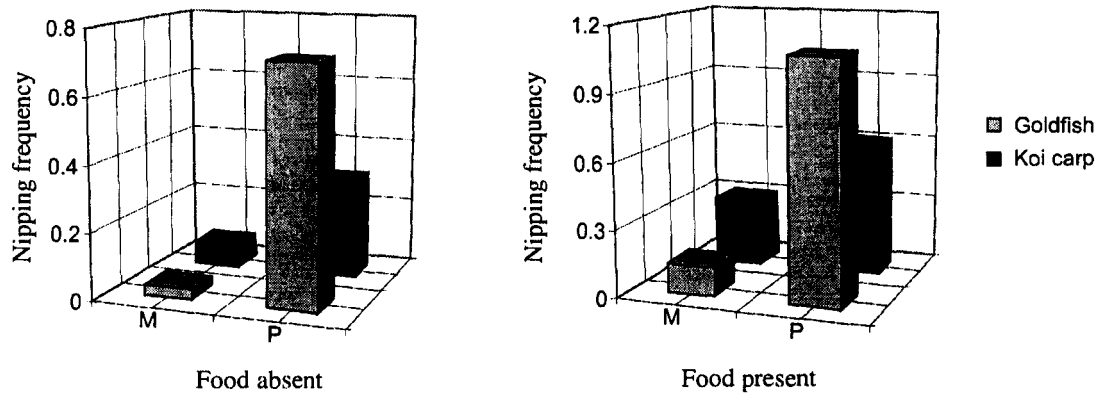

Fig. 3. Standardised (per capita) nipping frequency per trial estimated for koi carp and goldfish under monoculture (M) and polyculture (P) conditions in aquaria in the presence and absence of food (tubifex)

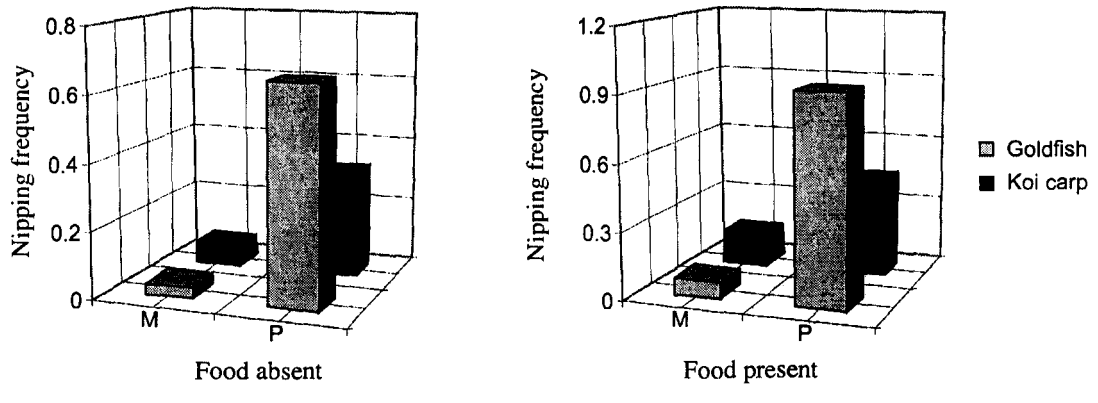

Fig. 4. Standardised (per capita) nipping frequency per trial estimated for koi carp and goldfish under monoculture (M) and polyculture (P) conditions in aquaria in the presence and absence of food (plankton)

al. (1997) observed that the prerequisites for competition exist when mixed populations of fish species are trapped in shrinking ponds during drought. In India, ornamental fish ponds are generally much smaller, compared to other aquaculture ponds, and competition pressure may severely affect the production status of the "non aggressive" species under such confined habitat conditions.

Although the present laboratory-based findings are not sufficient to be applied in a predictive way to judge interspecific interrelationships in ponds, they do illustrate behavioural mechanisms by which koi carp may negatively impact goldfish under confined conditions in ponds. A major limitation of the present study lies in its direct observation method allowing space for individual errors

and selective judgments. Nevertheless, the data are clearly indicative of behavioural interactions between the species concerned and further studies are encouraged to increase precision of observations (e.g. by video-recording and electronic image analysis systems). Further research is also required to investigate how dynamic behavioural interactions are affected by variation in abundance ratios as well as food availability and temperature, i.e. factors that play significant roles in tropical pond conditions.

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Comparison of food selection and growth performance of koi carp, *Cyprinus carpio* L., and goldfish, *Carassius auratus* (L.) in mono- and polyculture rearing in tropical ponds

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Abstract

To compare the effect of polyculture against conventional monoculture on ornamental carp production, investigations on food selection and growth performance of koi carp (K), *Cyprinus carpio* L. and goldfish (G), *Carassius auratus* (L.) were conducted in a 11-week rearing experiment in two monoculture (100% K and 100% G) and five polyculture (90% K–10% G, 70% K–30% G, 50% K–50% G, 30% K–70% G and 10% K–90% G) conditions in tropical ponds. There were three replicates for each treatment. Environmental conditions and food availability were similar in all the treatments. Ivlev's electivity index showed that both fish species avoided phytoplankton and preferred cladocerans to other zooplankton groups (copepods and rotifers) in monotypic conditions. However, in the polyculture treatments, the positive electivity of goldfish towards cladocerans reduced significantly ($P < 0.05$), while the percentage of copepods, rotifers and phytoplankton in the gut content increased. No significant differences in weight gain, specific growth rate and deformities were recorded at harvest for koi carp between the different treatments ($P > 0.05$). Even the survival rate of koi carp recorded above 90% in all the treatments. However, the goldfish recorded significantly better weight gain, specific growth rate and survival in monoculture (100% G), compared with the polyculture treatments ($P < 0.05$). Goldfish deformities were lowest ($P < 0.05$) in the monoculture treatment (2.42%). The number of marketable fish above a set size limit of 4 g total weight was significantly higher in the two monoculture treatments, compared with the five polyculture treatments ($P < 0.05$). Keeping in view of the dietary similarities of koi carp and goldfish, and the aggressive nature of koi carp in polyculture, it is suggested to refrain from polyculture of goldfish and koi carp until further documentations relating to optimum stocking density and management of polyculture of ornamental carps are available.

Keywords: koi carp, *Cyprinus carpio* L., goldfish, *Carassius auratus* (L.), pond polyculture, food selection, growth performance, marketable fish

Introduction

Polyculture is the only possible way of simultaneously producing more than one fish species from the same rearing space (Papoutsoglou, Petropoulos & Barbieri 1992; Papoutsoglou, Miliou, Karakatsouli, Tzitzinakis & Chadio 2001). The principle of polyculture is based on the fact that cultured fish species feed on different levels of food chain and environment (Milstein, Wahab & Rahman 2002). The productivity of the aquatic system is thus increased by more efficiently utilizing ecological resources within the environment. Stocking two or more complimentary fish species can increase the maximum standing

crop of a pond by allowing a wide range of available food items and the pond volume to be utilized (Lutz 2003).

Most of the literature available on the husbandry of ornamental fish suggests monoculture (Kestemont 1995; Asano, Ako, Shimizu & Tamaru 2003; Kaiser, Endemann & Paulet 2003; McGovern-Hopkins, Iwai & Tamaru 2003). One of the possible reasons could be the differences in the culture period for different fish species (Watson & Shireman 1996). While food fish producers can sell any amount of fish harvested, ornamental fish are sold by number and have to be of a minimum size to be accepted in the market (Olivier & Kaiser 1997). Some species have a market for small individuals, and the farmer may harvest the pond after only 8–10 weeks of grow-out. Others may require much longer culture periods. The extreme diversity of the industry prohibits gross generalizations in this area. Besides, frequent netting and sorting of fish of saleable size is not advisable, as it may cause damage to the fish.

Rapid increase in market demand during the past decade has shifted producers of ornamental fish in India from small-scale tank culture to large-scale fish culture in earthen ponds. Pond culture has also presented the opportunity to polyculture ornamental cyprinids, particularly species like koi carp, *Cyprinus carpio* L. and goldfish, *Carassius auratus* (L.), that require a similar culture period of 10–12 weeks to attain the marketable size, could be stocked together to optimize the utilization of available resources. At present, polyculture is being practiced on some ornamental fish farms. However, the stocking ratio and management methods are mostly determined by the respective farmer on an ad hoc basis, and vary from one farm to another. According to our knowledge, there is no documentation available on the growth and feeding habit of ornamental cyprinids under polyculture conditions.

The aim of the present study was to evaluate and compare the growth and food selection of koi carp and goldfish raised in monoculture and different polyculture combinations in ponds under almost similar environmental conditions and food availability.

Materials and methods

Two- to three-week old larvae of koi carp, *C. carpio* L. (0.12 ± 0.014 g) and goldfish, *C. auratus* (L.) (0.16 ± 0.018 g) were obtained from a local fish farm (Rainbow Ornamentals, Jalpaiguri, India), and acclimatized in 24 outdoor concrete tanks (capacity: 2000 L) for 1 week prior to the experiment. After the acclimatization period, fish of each species were divided into two batches. The first batch of fish was reared for 11 weeks (5 September–18 November, 2004) in 21 earthen ponds ($L \times W \times H$: 9.14 × 6.10 × 1.07 m; capacity: 59 600 L) in Raninagar village, Jalpaiguri, India. To study their growth, survival and food selection under monoculture and different polyculture regimes, seven treatments were examined: (1) koi carp monoculture (100% K); (2) 90% koi and 10% goldfish (90% K–10% G); (3) 70% koi and 30% goldfish (70% K–30% G); (4) 50% koi and 50% goldfish (50% K–50% G); (5) 30% koi and 70% goldfish (30% K–70% G); (6) 10% koi and 90% goldfish (10% K–90% G); and (7) goldfish monoculture (100% G).

There were three replicates for each treatment. The stocking density corresponded to 0.3 fish L^{-1}, as optimized earlier (Jha & Barat 2005a). Fish in each pond were fed by transferring about 1000 L of zooplankton water from a series of ponds culturing plankton. The introduction of exogenous plankton was found out to be more effective in maintaining better growth and survival rate of ornamental cyprinids compared with direct application of organic manure in the ponds, or supplemental feeding with commercial diets (Jha & Barat 2005b). During the daily introduction of plankton-rich water, about 1000 L of excess water was discharged from each pond. A plankton cloth was tied over the outflow water pipe to prevent any loss of zooplankton during the process. The ponds used for culturing plankton were fertilized with poultry manure at 0.26 kg m^{-3} at the beginning and subsequently once in every 10 days (Jha, Sarkar & Barat 2004).

Every week, four randomly selected fish of each species were removed from each pond at 11:00 hours and sacrificed for examination of food selection and food consumption. Routine examination procedures were followed for gut analysis (Jhingran, Natarajan, Banerjea & David 1988). The extent of prey selection by koi carp and goldfish were determined using Ivlev's formula (Ivlev 1961): $E = (r_1 - p_1)/(r_1 + p_1)$; where E is the electivity value, r_1 is the relative quantity of any ingredient in the gut expressed as percent and p_1 is the relative quantity of the same ingredient in the food complex, also expressed as percent. The application of this formula gives a range of values from +1.0 for a very high degree of selection to −1.0 for complete avoidance. A value of zero indicates that the prey is present in the diet in the same proportion as it is found in the environment, viz. complete absence of food selection.

The second batch of fish was maintained at a stocking density of 0.3 fish L^{-1} in seven outdoor cement tanks (capacity: 2000 L) under seven treatments of monoculture and polyculture combinations, similar to the first batch. About 30 L of plankton water were channeled into each tank daily for feeding the fish and a similar amount of excess water (30 L) was discharged. Fish cultured in these seven tanks were transferred to the experimental ponds corresponding to their respective treatments every week to substitute the fish of the first batch, which were captured for gut examination, to ensure that the results of the growth trial were not affected.

Water samples were collected from the ponds once weekly. The concentrations of nutrients (PO_4-P, NH_4-N, NO_3-N, NO_2-N) and routine water quality parameters (free CO_2, alkalinity, dissolved oxygen, biological oxygen demand, specific conductivity) were analysed according to methods as described by APHA (1998). pH was measured *in situ* using a portable pH meter (Hanna Instruments, Rua do Pindelo, Portugal). Temperature was recorded by a centigrade thermometer. Samples of plankton were collected in plankton net made of standard bolting silk cloth (No. 21 with 77 mesh cm^{-2}) twice a week. Collected plankton samples were concentrated to 20 mL, and preserved in 4% formalin. Enumerations of 1 mL of concentrated plankton were performed under a stereoscopic microscope using Sedgwick Rafter Counting Cell.

For growth rate determinations, individual fish weights were recorded both at the beginning and during harvest. Five hundred fish of each species were randomly collected from each pond and weighed individually to the nearest 0.001 g. For this, the fish were anaesthetized with 0.04 g L^{-1} of tricaine methane sulphonate (MS–222). Among these 500 fish, the number and percent of fish with body deformities were recorded. Dead fish were removed daily and were not replaced during the course of study, and differences between the number of fish stocked and the number of fish at harvest were used to calculate percent mortality in each treatment. Data were normalized using angular transformation (Mosteller & Youtz 1961). The specific growth rate (SGR; % day^{-1}) for each treatment was calculated as: $SGR = 100\ [(\ln W_t - \ln W_0)\ t^{-1}]$; where W_0 and W_t are the initial and final live weight of the fish (g), respectively, and t is culture period in days (Ricker 1975).

A one-way ANOVA procedure was performed to detect significant differences in water quality parameters as well as growth, survival, SGR and deformities in each fish species among treatments. A Tukey's test (Zar 1999) was used to compare and rank means. A level of significance of $P < 0.05$ was used. The number of marketable fish at the end of growth period was calculated using the function for a normal distribution curve, where $z = (y - \mu)/\sigma$; y is the lowest marketable weight (g), μ is the mean weight of the population, σ is the standard deviation of the total weight and z follows the standard normal probability distribution which determines the probability of finding fish above a given range. The number of marketable fish (n) was then determined using the table value of the normal probability distribution (P) as follows: $n = (1 - P)^* h$; h is total number of fish produced minus deformed or damaged fish.

Results

The plankton abundance and species diversity were similar in all the ponds, as they received daily plankton water from the same source. Cladocerans were in higher abundance in the environment (54.11%) compared with copepods (38.05%). Rotifers constituted 2.62% of total plankton composition, while phytoplankton comprised 5.21%. *Moina* and *Daphnia* were most abundant among cladocerans, while *Cyclops* were the most dominant copepod (Table 1).

Table 1 Species composition, average abundance (no. L^{-1}) and relative abundance (% of total numbers) of planktonic organisms present in the experimental ponds

Plankton	no. L^{-1}	%
Daphnia	314.20	17.04
Moina	389.15	21.11
Ceriodaphnia	218.07	11.83
Bosmina	76.17	4.13
Cladocera	997.59	54.11
Cyclops	380.24	20.62
Diaptomus	39.60	2.15
Nauplii	281.64	15.28
Copepoda	701.48	38.05
Brachionus	28.92	1.57
Keratella	19.39	1.05
Rotifera	48.31	2.62
Chlorella	18.35	0.99
Navicula	40.12	2.18
Spirogyra	21.07	1.14
Scenedesmus	1.32	0.07
Phacus	14.10	0.77
Synedra	1.19	0.06
Phytoplankton	96.15	5.21
Total plankton	1843.53	–

Table 2 Ivlev's electivity index applied to the gut contents of koi carp larvae along with percentage of planktonic organisms in the fish gut

	Treatment											
	100% K		90% K–10% G		70% K–30% G		50% K–50% G		30% K–70% G		10% K–90% G	
Plankton	% in gut	Ivlev's index	% in gut	Ivlev's index	% in gut	Ivlev's index	% in gut	Ivlev's index	% in gut	Ivlev's index	% in gut	Ivlev's index
Daphnia	28.29	0.248	30.14	0.278	29.70	0.271	31.22	0.294	30.18	0.278	30.51	0.283
Moina	29.51	0.166	30.96	0.189	27.39	0.129	28.09	0.142	29.79	0.171	32.16	0.207
Ceriodaphnia	16.98	0.179	15.29	0.125	17.51	0.194	14.52	0.102	17.53	0.194	12.32	0.021
Bosmina	8.84	0.363	9.65	0.401	10.38	0.431	12.04	0.489	8.29	0.335	12.05	0.489
Cladocera	83.62	0.214	86.04	0.228	84.98	0.222	85.87	0.227	85.79	0.226	87.04	0.233
Cyclops	9.13	−0.386	8.05	−0.438	10.16	−0.340	9.86	−0.353	8.68	−0.407	9.05	−0.390
Diaptomus	1.08	−0.331	0.95	−0.387	0.82	−0.448	0.21	−0.822	0.11	−0.903	0.16	−0.861
Nauplii	4.19	−0.569	4.05	−0.581	3.34	−0.641	3.78	−0.603	4.85	−0.518	3.63	−0.616
Copepoda	14.40	−0.451	13.05	−0.489	14.32	−0.453	13.85	−0.466	14.04	−0.461	12.84	−0.495
Brachionus	0.28	−0.697	0.11	−0.869	0.17	−0.805	0.10	−0.880	–	−1	–	−1
Keratella	0.65	−0.235	0.51	−0.346	0.34	−0.511	0.12	−0.795	0.07	−0.875	0.04	−0.927
Rotifera	0.93	−0.476	0.62	−0.617	0.51	−0.674	0.22	−0.845	0.07	−0.948	0.04	−0.970
Chlorella	0.14	−0.752	–	−1	–	−1	–	−1	–	−1	–	−1
Navicula	0.50	−0.627	0.21	−0.824	0.14	−0.879	0.06	−0.946	0.10	−0.912	0.08	−0.929
Spirogyra	0.32	−0.562	0.08	−0.869	0.05	−0.916	–	−1	–	−1	–	−1
Scenedesmus	–	−1	–	−1	–	−1	–	−1	–	−1	–	−1
Phacus	0.09	−0.791	–	−1	–	−1	–	−1	–	−1	–	−1
Synedra	–	−1	–	−1	–	−1	–	−1	–	−1	–	−1
Phytoplankton	1.05	−0.665	0.29	−0.894	0.19	−0.929	0.06	−0.977	0.10	−0.962	0.08	−0.969

Treatments represent monoculture of koi carp (100% K) to various combinations of polyculture with goldfish (G).

In both fish species, cladocerans were relatively higher in diet abundance, ranging from 83.62% (100% K) to 87.04% (10% K–90% G) for koi carp (Table 2), and 66.62% (90% K–10% G) to 94.62% (100% G) for goldfish (Table 3). The copepods, on the other hand contributed only 12.84% (10% K–90% G) to 14.40% (100% K) in koi carp gut (Table 2), and 5.38% (100% G) to 29.93% (90% K–10% G) in goldfish gut (Table 3).

The results of the electivity index estimate revealed that both species of fish preferred cladocerans in all treatments. Electivity towards other zooplankton groups (copepods and rotifers) and phytoplankton were negative for both the cyprinids. However, for goldfish, the values for the positive electivity towards cladocerans differed significantly ($P < 0.05$) from one treatment to another, ranging from 0.104 (90% K–10% G) to 0.272 (100% G). Likewise, the values of the negative electivity towards the copepods also differed significantly ($P < 0.05$) among the goldfish stocked in the various treatments (Table 3). However, for the koi carp, the levels of the positive selection of cladocerans, or negative selection of copepods, as evidenced from the electivity indices (Table 2) did not differ significantly between the different treatments ($P > 0.05$).

Water temperature was between 19 and 30 °C during the 11-week growth period. However, there was no difference in the water temperature between the different treatments on any particular sampling date. The 100% K and 90% K–10% G treatments recorded significantly lower values of dissolved oxygen ($P < 0.05$), compared with all other treatments (Table 4). The range of the recorded pH values was also lower in these two treatments. Significantly higher ($P < 0.05$) values of free CO_2 and total alkalinity were also recorded in 100% K and 90% K–10% G than all other treatments. Highest values of BOD and NO_3-N were recorded in 100% K ($P < 0.05$). However, there were no significant differences in the values of specific conductivity, NO_2-N, NH_4-N and PO_4-P, recorded in the different treatments ($P > 0.05$; Table 4).

At harvest, there were no significant differences in the weight gain, SGR and the percentage of deformed fish estimated for koi carp among the various treatments ($P > 0.05$; Table 5). The survival rate of koi carp ranged from 92.59% (30% K–70% G) to 95.70% (90% K–10% G). However, polyculture had a significant effect on the growth parameters of goldfish (Table 5) and the highest weight gain (9.36 g) was recorded in the monoculture treatment (100% G). The survival rate of goldfish was significantly higher

Table 3 Ivlev's electivity index applied to the gut contents of goldfish larvae alongwith percentage of planktonic organisms in the fish gut

	Treatment											
	90% K–10% G		70% K–30% G		50% K–50% G		30% K–70% G		10% K–90% G		100% G	
Plankton	% in gut	Ivlev's index	% in gut	Ivlev's index	% in gut	Ivlev's index	% in gut	Ivlev's index	% in gut	Ivlev's index	% in gut	Ivlev's index
Daphnia	19.04	0.055	19.18	0.059	20.01	0.080	22.31	0.134	27.02	0.226	35.10	0.346
Moina	20.12	−0.024	20.56	−0.013	21.86	0.017	22.78	0.038	23.37	0.051	28.25	0.143
Ceriodaphnia	15.01	0.118	14.38	0.113	15.47	0.133	17.94	0.205	16.08	0.152	15.10	0.121
Bosmina	12.45	0.502	13.06	0.519	12.69	0.509	14.23	0.550	15.18	0.572	16.17	0.593
Cladocera	66.62	0.104	67.18	0.108	70.03	0.128	77.26	0.176	81.65	0.203	94.62	0.272
Cyclops	16.27	−0.118	15.70	−0.135	15.21	−0.151	12.37	−0.250	10.14	−0.341	2.08	−0.817
Diaptomus	2.05	−0.024	2.34	−0.066	1.85	−0.075	1.82	−0.083	1.38	−0.218	0.18	−0.845
Nauplii	11.61	−0.136	11.29	−0.150	10.12	−0.203	7.55	−0.338	6.32	−0.415	3.12	−0.661
Copepoda	29.93	−0.119	29.33	−0.129	27.18	−0.166	21.74	−0.273	17.84	−0.362	5.38	−0.752
Brachionus	0.64	−0.421	0.65	−0.414	0.38	−0.610	0.28	−0.697	0.14	−0.836	–	−1
Keratella	0.75	−0.166	0.68	−0.214	0.70	−0.200	0.26	−0.603	0.18	−0.707	–	−1
Rotifera	1.39	−0.307	1.33	−0.327	1.08	−0.416	0.54	−0.658	0.32	−0.782	–	−1
Chlorella	0.30	−0.535	0.35	−0.478	0.31	−0.523	0.05	−0.904	–	−1	–	−1
Navicula	0.81	−0.458	0.88	−0.425	0.63	−0.552	0.19	−0.840	0.09	−0.921	–	−1
Spirogyra	0.74	−0.213	0.60	−0.310	0.57	−0.333	0.06	−0.900	–	−1	–	−1
Scenedesmus	–	−1	–	−1	–	−1	–	−1	–	−1	–	−1
Phacus	0.21	−0.571	0.33	−0.400	0.20	−0.588	0.16	−0.656	0.10	−0.770	–	−1
Synedra	–	−1	–	−1	–	−1	–	−1	–	−1	–	−1
Phytoplankton	2.06	−0.433	2.16	−0.414	1.71	−0.506	0.46	−0.838	0.19	−0.930	–	−1

Treatments represent monoculture of goldfish (100% G) to various combinations of polyculture with koi carp (K).

Table 4 Mean ± SE of selected water quality parameters analysed for the seven treatments at weekly intervals during the 11-week growth period

	Treatment						
Parameters	100% K	90% K – 10% G	70% K – 30% G	50% K – 50% G	30% K – 70% G	10% K – 90% G	100% G
pH*	5.5–7.4	5.5–7.4	6.0–8.1	6.5–7.9	6.4–8.1	6.4–8.0	6.6–7.8
Dissolved oxygen ($mg\,L^{-1}$)	5.75 ± 0.28^{b}	5.72 ± 0.09^{b}	5.92 ± 0.14^{ab}	6.27 ± 0.31^{a}	6.20 ± 0.16^{a}	6.24 ± 0.19^{a}	6.31 ± 0.28^{a}
Free CO_2 ($mg\,L^{-1}$)	2.92 ± 0.09^{a}	2.86 ± 0.14^{a}	2.60 ± 0.08^{b}	2.51 ± 0.12^{b}	2.58 ± 0.11^{b}	2.62 ± 0.16^{b}	2.59 ± 0.14^{b}
BOD ($mL\,L^{-1}$)	1.31 ± 0.04^{a}	1.19 ± 0.08^{ab}	1.20 ± 0.06^{ab}	1.08 ± 0.05^{bc}	0.95 ± 0.10^{c}	1.05 ± 0.09^{c}	1.01 ± 0.05^{c}
Total alkalinity ($mg\,L^{-1}$)	38.26 ± 2.19^{a}	37.10 ± 1.87^{a}	30.04 ± 1.04^{bc}	28.19 ± 0.72^{c}	31.12 ± 1.29^{bc}	30.60 ± 0.88^{bc}	33.92 ± 1.40^{b}
PO_4-P ($mg\,L^{-1}$)	0.32 ± 0.041^{a}	0.32 ± 0.035^{a}	0.30 ± 0.031^{a}	0.29 ± 0.034^{a}	0.27 ± 0.028^{a}	0.29 ± 0.016^{a}	0.28 ± 0.028^{a}
NH_4-N ($mg\,L^{-1}$)	0.167 ± 0.030^{a}	0.165 ± 0.021^{a}	0.168 ± 0.014^{a}	0.152 ± 0.026^{a}	0.155 ± 0.029^{a}	0.152 ± 0.022^{a}	0.155 ± 0.021^{a}
NO_2-N ($mg\,L^{-1}$)	0.015 ± 0.004^{a}	0.015 ± 0.005^{a}	0.013 ± 0.001^{a}	0.012 ± 0.003^{a}	0.013 ± 0.002^{a}	0.012 ± 0.003^{a}	0.011 ± 0.002^{a}
NO_3-N ($mg\,L^{-1}$)	0.160 ± 0.018^{a}	0.142 ± 0.014^{b}	0.135 ± 0.015^{b}	0.116 ± 0.012^{c}	0.125 ± 0.013^{bc}	0.128 ± 0.011^{bc}	0.121 ± 0.012^{c}
Specific conductivity ($mhos\,cm^{-1}$)	0.26 ± 0.013^{a}	0.25 ± 0.012^{a}	0.23 ± 0.014^{a}	0.21 ± 0.029^{a}	0.23 ± 0.032^{a}	0.24 ± 0.014^{a}	0.23 ± 0.018^{a}

*For pH, the range of recorded values are presented.

Data in the same row with different superscripts are significantly different ($P < 0.05$).

Table 5 Growth performance estimated in koi carp and goldfish after mono and polyculture rearing for 11 weeks

	Treatment						
	100% K	90% K–10% G	70% K–30% G	50% K–50% G	30% K–70% G	10% K–90% G	100% G
Koi carp							
Final weight (g)	8.05 ± 0.09^{a}	8.09 ± 0.18^{a}	7.95 ± 0.12^{a}	7.90 ± 0.18^{a}	7.90 ± 0.31^{a}	7.88 ± 0.14^{a}	–
Mean growth increment (g)	7.93 ± 0.09^{a}	7.97 ± 0.18^{a}	7.83 ± 0.12^{a}	7.78 ± 0.18^{a}	7.78 ± 0.31^{a}	7.76 ± 0.14^{a}	–
SGR (% day^{-1})	5.45 ± 0.09^{a}	5.47 ± 0.18^{a}	5.44 ± 0.12^{a}	5.44 ± 0.18^{a}	5.44 ± 0.31^{a}	5.43 ± 0.14^{a}	–
Survival (%)	95.50^{a}	95.70^{a}	94.44^{ab}	94.11^{ab}	92.59^{b}	92.73^{b}	–
Deformed individuals (%)	1.91^{a}	2.02^{a}	1.73^{a}	2.04^{a}	2.26^{a}	1.78^{a}	–
Goldfish							
Final weight (g)	–	3.65 ± 0.21^{c}	3.80 ± 0.24^{c}	3.87 ± 0.22^{c}	4.01 ± 0.28^{c}	6.44 ± 0.22^{b}	9.53 ± 0.31^{a}
Mean growth increment (g)	–	3.48 ± 0.21^{c}	3.63 ± 0.24^{c}	3.70 ± 0.22^{c}	3.84 ± 0.28^{c}	6.27 ± 0.22^{b}	9.36 ± 0.31^{a}
SGR (% day^{-1})	–	3.97 ± 0.21^{e}	4.03 ± 0.24^{d}	4.05 ± 0.22^{d}	4.10 ± 0.28^{c}	4.71 ± 0.22^{b}	5.21 ± 0.31^{a}
Survival (%)	–	54.36^{f}	60.70^{e}	65.51^{d}	71.90^{c}	81.07^{b}	91.41^{a}
Deformed individuals (%)	–	11.53^{a}	8.93^{b}	7.07^{c}	4.23^{d}	3.17^{de}	2.42^{e}

Different superscripts in a row represent statistically significant differences ($P < 0.05$).

Table 6 The average number of marketable koi carp (K) and goldfish (G) (those heavier than 4.0 g) produced, together with marketable fish produced expressed as a percentage of total number of fish produced* (A) and as a percentage of number of fish stocked (B) in the different treatments

	Number of fish stocked (fish pond^{-1})			Number of marketable fish produced* (fish pond^{-1})			Marketable fish (%)	
Treatment	K	G	Total	K	G	Total	(A)*	(B)
100% K	17880	–	17880	16735	–	16735^{a}	100^{a}	94.0^{a}
90% K–10% G	16092	1788	17880	15078	36.61	15114.61^{ab}	95.16^{b}	84.53^{c}
70% K–30% G	12516	5364	17880	11602	561.87	12163.87^{c}	84.59^{c}	68.03^{e}
50% K–50% G	8940	8940	17880	8231	1448.57	9679.57^{d}	71.94^{d}	54.14^{f}
30% K–70% G	5364	12516	17880	4846	4355.66	9201.66^{d}	69.10^{e}	51.46^{g}
10% K–90% G	1788	16092	17880	1626	12536	14162^{bc}	100^{a}	79.21^{d}
100% G	–	17880	17880	–	15909	15909^{ab}	100^{a}	88.98^{b}

*Excluding deformed fish.

Different superscripts in a column represent statistically significant differences ($P < 0.05$).

($P < 0.05$) in the monoculture treatment (91.41%), compared with the different polyculture treatments (Table 5). The percentage of goldfish deformities was highest in 90% K–10% G, followed in decreasing order by 70% K–30% G, 50% K–50% G, 30% K–70% G, 10% K–90% G and 100% G treatments ($P < 0.05$; Table 5).

To determine the output of marketable fish, the percentage and number of fish above a total weight of 4 g (excluding deformed individuals) was estimated from the probability distribution at the end of the study (Table 6). Polyculture had a direct effect on fish production as the monoculture treatments for each species produced the highest percentage of marketable fish (Table 6). Among the various polyculture treatments, 90% K–10% G and 10% K–90% G produced a significantly greater number and percentage of marketable fish ($P < 0.05$), compared to other treatments (Table 6). All the fish harvested in the 100% K, 100% G and 10% K–90% G treatments (excluding deformed fish) attained marketable size (Table 6).

Discussion

The similarity in the types of organisms present in the gut of koi carp and goldfish may be because of the fact that all ponds were maintained under similar management conditions and the plankton water was

supplied from a series of ponds, also similar in size and management. Hence there was no difference in food resource between the experimental ponds. Water quality was quite similar in all the treatments. Lower pH and dissolved oxygen in 100% K and 90% K–10% G treatments may be explained by koi carp stirring up mud from the bottom. Although earlier reports on koi carp are lacking, other carp species are known to create management problems in fish ponds by stirring up pond bottoms, thereby releasing nutrients from the soil (Wahab, Rahman & Milstein 2002), creating turbidity and lowering dissolved oxygen (Lutz 2003).

Goldfish showed better weight gain in monoculture, compared with polyculture treatments, while the koi carp recorded no significant differences in growth between different treatments. Within the different polyculture treatments, the highest growth for goldfish was recorded in 10% K–90% G. Polyculture had a negative effect on goldfish production, however, absence of any earlier report relating to polyculture of coloured cyprinids in tropical pond conditions makes it difficult to draw conclusions about factors responsible for this reduced growth rate and production. One of the possible reasons could be differences in the food selection between the two species, and competition for food between them under polyculture conditions.

The koi carp diet in the monoculture treatment (100% K) consisted of 12 genera of plankton, which was reduced to fewer species in the different polyculture treatments. A rejection of phytoplankton was observed in all treatments, and is consistent with earlier findings (Jha & Barat 2005b). However, four genera of phytoplankton were identified in koi carp intestines collected from 100% K, compared to two genera in 90% K–10% G and 70% K–30% G, and one in each of the 50% K–50% G, 30% K–70% G and 10% K–90% G treatments. Greater diversity of food in the guts of monotypic populations may suggest that segregation by cohabiting fish species results in consumption of a narrower range of food items in polytypic communities, than when only one species is present (Andrusak & Northcote 1971; Clady 1981). Being the more aggressive species in polyculture, koi carp could select its preferred group of plankton (cladocera) within this narrow range, and the electivity towards cladocerans recorded similar in all the treatments.

In goldfish, phytoplankton was absent and only six genera of zooplankton were found in the intestines of monotypic populations (100% G). The plankton diversity significantly increased ($P < 0.05$) in the gut content of polycultured goldfish communities (Table 3). However, greater diversity of the diet does not necessarily suggest better feeding conditions, as fish populations often consume a greater variety of food items under adverse conditions than when food supplies are unlimited (Ivlev 1961). Greater diversity of plankton from the guts of polycultured goldfish may be influenced by the consumption of their preferred food (cladocera) by the koi carp. In monoculture, goldfish recorded a very strong selection for the cladoceran, *Daphnia* (0.346) and the genera contributed 35.10% of the gut contents in 100% G, which was significantly reduced in polycultured goldfish ($P < 0.05$), recording only 19.04% in goldfish gut content in 90% K–10% G, with an electivity of 0.055. Simultaneously, there was an increased consumption of copepods, rotifers and phytoplankton in polyculture compared to monoculture.

Behavioural observations in an earlier experiment showed that koi carp were more active in polyculture treatments, compared with monoculture (Jha, Jha, Pal & Barat 2005). Similar aggressive behaviour of polycultured koi carp was also noted during the present study and could account for the increased rate of deformities and fin damages observed in goldfish in the polyculture treatments. However, it was not possible to document details of the interspecific interactions between koi carp and goldfish in pond conditions. The deformities in both species were mostly scoliosis, spinal and caudal abnormalities and bent fins, and could not have been induced environmentally, as the management conditions of all the treatments were similar. Ornamental fish must be visually attractive to be marketable and deformed or damaged fish are not saleable, even if they attain marketable size.

The main tool for managing polyculture systems and maximizing fish production is the knowledge of fish–fish and fish–environment quantitative relationships (Milstein 1992). One problem frequently encountered in polyculture involves the overlap of food or habitat preferences among species or antagonism (Lutz 2003). Brummett and Alon (1994) indicated that although growth of redclaw crayfish *Cherax quadricarinatus* (VonMartens) was not adversely affected by the presence of nile tilapia, *Oreochromis niloticus* (L.) in polyculture, tilapia growth and food conversion were significantly impacted by redclaw crayfish. Yashouv (1968) related similar problems encountered in polyculture of tench, *Tinca tinca* (L.) and common carp, *C. carpio* L. In spite of feed being ap-

plied to ponds, the two species apparently competed for the same resources and carp production was reduced when tench was present. Studies on stable carbon and nitrogen isotope values from ponds polyculturing silver carp, *Hypophthalmichthys molitrix* (Val.) and bighead carp, *Aristichthys nobilis* (Rich.) in China suggested that there were various degrees of dietary overlap between the two species with an average of 60% of their food from the same trophic level (Gu, Schell, Huang & Yie 1996). In another experiment, Mattson (1998) recorded similar feeding preferences in *Oreochromis shiranus* (Boulenger) and *Barbus paludinosus* (Peters), when offered an array of planktonic food in aquaria. In a study on polyculture of tench, common carp and bigmouth buffalo, *Ictiobus cyprinellus* (Val.), Adamek, Sukop, Rendon and Kouril (2003) observed food competition between tench and carp (60.8%) and between tench and bigmouth buffalo (47.4%). Vromant, Nam and Ollevier (2002) recorded significant interspecific competition between nile tilapia and common carp in polyculture systems in intensively cultivated rice fields. Preliminary data available on koi carp and goldfish suggested that both species are likely to prefer the bottom tank level (Sandford 1998) and eat similar food items (Axelrod & Vorderwinkler 1970).

Another problem with polyculture wherever labour costs are relatively high involves handling and sorting species at harvest. In our experiment, every pond was netted three times at harvest, which could have aggravated body and fin damages, particularly in goldfish. According to Milstein (1992), polyculture is the appropriate technique when the goal is production of low-cost fish. When the goal is production of more expensive fish, monoculture simplifies the management (Wohlfarth & Schroeder 1979; Hepher & Pruginin 1981). The financial risks associated with each species in the different combinations requires evaluation (Milstein 1992).

Polyculture had a direct effect on the number of marketable fish. Monoculture treatments yielded the largest number of saleable fish. In contrast to food fish production, where the total number of fish produced determines productivity, ornamental fish can only be sold once they have reached a particular size (4 g or more). From the present study, a diminishing return becomes apparent in ornamental carp polyculture, compared to monoculture. Besides, keeping in view of the dietary similarities of koi carp and goldfish, and the aggressive nature of koi carp in polycuture, possibly leading to increased deformities and lower weight gain and SGR of polycultured goldfish communities, it is suggested to refrain from polyculture of goldfish and koi carp until further results relating to optimal stocking density and management of polyculture of ornamental carps are available.

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