

Comparison of Five Different Practical Diets with Various Concentrations of Dietary Protein in Nursery Ponds: Survival and Growth of Indian Major Carp Fry

K. SINGH, S.K. GARG*, A. BHATNAGAR and A. KALLA

*Laboratory of Aquaculture Management
Department of Zoology and Aquaculture
CCS Haryana Agricultural University Hisar-125004
Indi*

Abstract

The purpose of this study was to evaluate the existing and modified experimental diets for use in nursery ponds for feeding the Indian major carp fry. Fry (mean body weight 1.11 ± 0.06 - 1.18 ± 0.01 g) were stocked (@ 3.2 lacs ha⁻¹) in nurseries in August and fed on one of the five formulated diets (Protein contents ranged between 28 to 35%) over a period of 40 days. Lowest survival, growth and SGR were observed in ponds (treatments 1 and 2) where the fry were fed on traditional diets. Studies have further revealed that survival and growth (weight and length) of the fry increased, while FCR decreased with each increase in the dietary protein, fat and grass energy contents. An increase in the inclusion levels of full fat processed soybean for obtaining high protein and energy, not only improved fry survival and growth, but also diminished ammonia pollution in nurseries. An investigation on the effects of feeds on water quality parameters have revealed that nutrients (o-Po₄, total kjeldhal nitrogen, No₃-N), alkalinity and turbidity and all the productivity indicating parameters viz. chlorophyll 'a', net primary productivity, including plankton population, their species diversity increased with each increase in the dietary protein contents and thus highest values coincided with the highest survival and growth. An analysis of bottom sediment have revealed that nutrients (o-Po₄ and No₃-N), alkalinity and benthic population also followed a similar trend. Multivariate analysis (Prien et al. 1993) of the data also revealed a significant ($P < 0.05$) positive correlation of nutrients, NPP and plankton population with fry growth. These studies thus indicated that the traditional diets used by the farmers are nutritionally inadequate. Hence to obtain high survival and growth in high density stocking, farmers may have to feed the fry on supplementary diets containing high protein contents (< 35%) preferably of plant origin supplemented with micronutrients and amino acids.

Introduction

Indian major carp fingerlings are in great demand all over the Indian sub-continent to serve as the stocking material for culturing table sized fish.

Corresponding author

Hence, fry rearing is one of the important phases and aims at obtaining high survival and growth for the production of fingerlings required for stocking. The success of any culture trial with fry depends largely on the presence of sufficient, preferred small food organisms the zooplankton (Alikunhi 1952, Alikunhi and Ramachandran 1955, Mitra and Mohapatra 1956). Pond fertilization with organic and inorganic fertilizers is a very cheap and effective method of increasing primary productivity, but their excessive use deteriorates the water quality (Boyd 1992, Garg and Bhatnagar 1996) and depletes the dissolved oxygen to detrimental level.

Pond farming at high intensity levels frequently limits the effects of fertilization (See Steffens 1990 for references). Further, high stocking rates also leads to the reduction of standing crop of natural fish food organisms to such a degree as to these organisms really cannot develop in larger scale even in case of vigorous production.

Hence in order to supplement the natural feed, supplementary compounded feeds of full physiological value in nurseries are required for obtaining high survival and growth in high density culture. The most common feed ingredients given to the fish in this region are the rice-bran and oilcakes (Jhingran and Pullin 1988). These diets neither contain essential nutrients in sufficient quantity nor optimum protein levels. Thus there is a need to formulate cheap, eco-friendly and nutritionally balanced diets for obtaining high survival and growth of Indian major carp fry in high density culture.

Most of the studies on protein requirements have been conducted under laboratory conditions, which indicate high protein requirements for carps (Das and Ray 1991). Since the present studies were conducted under field conditions, where the ponds were fertilized with organic fertilizers, hence diets containing 28 to 35% crude protein were formulated. The aim of the present investigations, therefore were to study and compare the efficacy of five different practical diets on survival and growth performance of Indian major carp fry. An attempt has also been made to evaluate the physico-chemical and biological characteristics of the pond water and sediment in relation to fry survival and growth.

Materials and Methods

Pond facilities and experimental design

The experiment was carried out in earthen ponds (area c.a. 0.125 to 0.25 ha, mean water depth 1 m) at the fish farm facility of M/s Royal Fish Farm, Hisar (Lat. 29°, 10'N; Long 75°, 46'E), for a period of 40 days from August to October, 2000. Prior to the commencement of treatments, the ponds were cleaned, lime was applied @ 200 kg·ha⁻¹ and filled with tubewell water (0.46 to 0.6‰ salinity) and were allowed to stabilize for about two weeks. Water levels were maintained (1 m depth) by replenishing the water daily from the tubewell. Pond fertilization, supplementary feeding and stock-

ing density (32 m²) were the various management practices adopted in these studies, their rates and frequency are given in table 1.

Experimental feeds and feeding schedule

Five different feeds were prepared (Table 2), ingredients were thoroughly ground, mixed and soaked overnight in water. Fish fry were fed daily in the morning (between 08⁰⁰ and 09⁰⁰) @ 6% BW. Fry were acclimated to the reference diet (Diet 1) for a period of one week, prior to the initiation of feeding trials. Fry (100 to 200) from each treatment were bulk weighed at weekly intervals to adjust the feeding rates and to calculate food conversion ration (FCR).

Proximate analysis

Proximate analysis of the ingredients and diets was carried-out following AOAC (1995). It indicated that protein contents of the diets (1-5) varied from 28 to 35% (Table 2). Ash contents were high in diet containing fish meal (diet-2), whereas fat and gross energy values were high in diets 4 and 5 containing 34 to 35% protein.

Stocking

Two replicates for treatment were mentioned and two weeks after the application of organic fertilizers, 10 day old Indian major carp fry viz. *Catla catla* (mean body wt. 1.45 ± 0.07 g, length 2.1 cm), *Labeo rohita* (mean body weight 1.17 ± 0.05 g, length 2.0 cm) and *Cirrhinus mrigala* (mean body weight 1.07 ± 0.02, length 1.71 cm) were stocked (@ 3.2 lacs·ha⁻¹) during August (Table 1).

Water quality monitoring

Water samples for the determination of water quality parameters were obtained in replicates of four before sunrise from each treatment at 20 day interval. Water temperature was recorded daily using digital thermometer. Dissolved oxygen (DO), pH, conductivity and salinity were measured using multiline F-set 3 (E. Merck Ltd., Germany). Turbidity was measured using Nephelo turbidity meter (Systronics) in NTU. All other parameters were determined following APHA (1998).

Biotic communities

For qualitative and quantitative estimations of phyto and zooplankton, water samples were collected in replicates of four from each treatment at 20 days interval. Plankton samples were collected by passing 10 l of water taken from five different locations of each treatment through a plankton net (mesh size 125 µm). The concentrated samples were then carefully

transferred to a measuring cylinder and made up to a volume of 40 ml with distilled water. Samples were preserved in small plastic bottles with 5% buffered formalin. Plankton densities were estimated using a Sedgewick Rafter counting cell (Wetzel and Likens 1979) under a binocular microscope.

Table 1. Experimental protocol

Sr. No.	Dietary treatments	Area of ponds	Stocking density (hectares)	Dosage of fertilizers	
				Cowdung (kg·w ⁻²)	Poultry (kg·w ⁻²)
1.	Mustard oil cake: Ricebran: MPA: 60:39:1	0.25	80000	104.16	31.25
2.	Mustard oil cake: Ricebran: Fish meal: MPA: 60:29:10:1	0.25	80000	104.16	31.25
3.	Mustard oil cake: Ricebran: Processed soybean: MPA: 60:29:10:1	0.25	80000	104.16	31.25
4.	Mustard oil cake: Ricebran: Processed soybean: MPA: 60:24:15:1	0.125	40000	52.08	15.62
5.	Mustard oil cake: Ricebran: Processed soybean: MPA: 60:19:20:1	0.125	40000	52.08	15.62

All ponds were fertilized at biweekly intervals using cow-dung @ 10,000 kg ha⁻¹y⁻¹ and poultry excreta @ 3,000 kg ha⁻¹y⁻¹

Rate of feeding @ 6% BWd⁻¹, Duration of experiment 40 days, Stocking density 3.2 lacs ha⁻¹,

Species stocked: *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*

MPA: Each kg contain Copper 312 mg, Cobalt 45 mg, Magnesium 2.114g, Iron 979 mg, Zinc 2.130 g, Iodine 156 mg, DL-Methionine 1.920 g, L-Lysine Mono Hydrochloride 4.4 g, Calcium 30%, Phosphorus 8.25%

Table 2. Ingredient contents (%) and proximate analysis (% dry weight basis) of experimental diets (1-5)

	Diets				
	1	2	3	4	5
Ingredients					
Mustard oil cake	60.00	60.00	60.00	60.00	60.00
Rice bran	39.00	29.00	29.00	24.00	19.00
Fish meal	-	10.00	-	-	-
Processed full fat soybean ^a	-	-	10.00	15.00	20.00
Mineral premix with amino acids ^b (MPA)	1.00	1.00	1.00	1.00	1.00
Proximate analysis (%)					
Dry matter	90.90	91.38	91.85	92.37	92.50
Crude protein	28.00	32.62	32.00	33.76	35.00
Crude fat	9.87	9.72	10.80	11.16	11.36
Crude fibre	12.76	10.87	11.12	10.54	10.04
Ash	9.79	11.28	8.46	7.57	8.41
NFE	30.40	26.89	29.24	29.34	27.53
Gross energy K J g ⁻¹	15.75	16.17	16.90	17.42	17.52

^aProcessed soybean: Soybean was hydrothermically processed in an autoclave at 121°C (15 Lbs) for 15 minutes to remove anti nutrient factors (ANFs)

^bEach kg contain: Copper 312 mg, Cobalt 45 mg, Magnesium 2.114g, Iron 979 mg, Zinc 2.130 g, Iodine 156 mg, DL-Methionine 1.920 g, L-Lysine Mono Hydrochloride 4.4 g, Calcium 30%, Phosphorus 8.25%

Identification of planktons to genus level was carried out using suitable keys and monographs (Ward and Whipple 1959, Needham and Needham 1963).

Net and gross primary productivity were determined on the sampling days by light and dark bottle technique (APHA 1998). Water samples for chlorophyll 'a' ($\text{mg}\cdot\text{l}^{-1}$) determination were also collected at 20 days interval. A known amount of water was filtered through Whatman filter paper (No. 40) and extracted using acetone (APHA 1998).

Phytoplankton and zooplankton species diversity (d) were determined using the diversity index of Shannon and Weaver (Washington 1984).

$$\bar{d} = -\sum ni/N \log_2 ni/N$$

where

n_i = number of individuals of i th species

N = total number of individuals

Analysis of bottom sediment

Sediment samples from the pond bottom were collected in duplicate on day 20 and day 40 using cone sampler (Area 858.3 sq cm). The samples were analyzed for electrical conductivity (EC), pH, alkalinity, hardness, o-Po_4 , $\text{No}_3\text{-N}$, chlorides and organic matter according to Piper (1950). The o-Po_4 , $\text{No}_3\text{-N}$ and organic matter were determined by preparing an extract of the air dried sediment sample, while for the determination of pH, EC, alkalinity, hardness and chlorides, a suspension of the moist sediment (1:10 w/v) sample was prepared.

For the study of macrozoobenthos, the mud samples from each pond were separated using 0.5 mm mesh size sieve (Hovgaard 1973). organisms were sorted out manually using a pair of forceps for qualitative and quantitative analysis. Species diversity was also calculated using Shannon and Weaver's diversity index (Washington 1984).

Statistical analysis

Data were subjected to ANOVA and multivariate analysis following Prien et al. (1993). Duncan's multiple range test (Duncan 1955) was also applied to find out the significant differences in fish growth, if any, between different treatments.

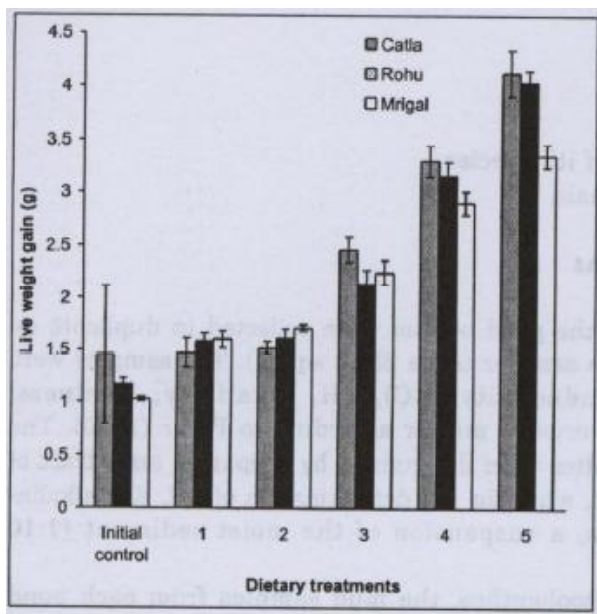
Results

Fish growth/biomass

Mortality in different treatments varied between 7 to 17.8 %. Fry in five different treatments were fed on one of the five formulated diets

(Table 3). ANOVA revealed that irrespective of the species stocked, a significant ($P < 0.05$) decrease in FCR, increase in mean live weight gain (Fig. 1), increase in mean length (cm), growth per cent gain in body weight and SGR were observed in fry fed on a diet (Treatment No. 5) containing the highest levels of protein (35%), while significantly ($P < 0.05$) high FCR, low survival and growth was observed in fry fed on traditional diets composed of MOC and ricebran and also when used in combination with fish meal (Treatments 1 and 2).

Physico-chemical characteristics of pond water



Dissolved oxygen (DO) remained at optimal levels in all the treatments and appeared to increase from treatments 1 to 5 (6.0 ± 0.19 to 7.56 ± 0.03). BOD was not high in any of the treatment and fluctuated between 2.80 ± 0.28

Fig. 1. Effect of five different dietary treatments (1-5) on mean (\pm S.E. mean) live weight gain (g) in Indian major carp fry (Catla, rohu and mrigal) under field conditions: 1 = 28.1% protein; 2 = 32.6% protein; 3 = 32.2% protein; 4 = 33.7% protein; 5 = 35.15% protein

Table 3. Effect of supplementary diets (1-5) on growth, weight gain and survival of Indian major carp fry under field conditions

Dietary treatments	Initial fish stock		Final fish stock (after 40 days)			Weight increase		SGR	FCR
	Species stocked	Initial mean weight (g)	Survival (%)	Final mean weight (g)	Final mean length (cms)	Live weight gain (g)	Growth % gain in (BW)		
1	Catla	1.45±0.06		2.92D±0.12	5.5±0.1	1.47D±0.14	103.42D±12.89	1.75C±0.16	3.46
	Rohu	1.17±0.05	82	2.75D±0.07	5.4±0.1	1.58D±0.08	138.17C±12.23	2.15D±0.12	
	Mrigal	1.03±0.02		2.63D±0.07	5.2±0.1	1.60D±0.08	155.76D±10.67	2.34D±0.10	
2	Catla	1.45±0.06		2.97D±0.40	5.5±0.1	1.52D±0.06	106.60D±9.10	1.80C±0.11	2.76
	Rohu	1.17±0.05	84	2.78D±0.07	5.1±0.07	1.61D±0.09	140.67C±13.72	2.18D±0.14	
	Mrigal	1.03±0.02		2.70D±0.04	5.3±0.1	1.71D±0.03	166.02D±4.38	2.41D±0.04	
3	Catla	1.45±0.06		3.92C±0.10	6.5±0.1	2.47C±0.12	173.01C±14.15	2.49B±0.13	2.03
	Rohu	1.17±0.05	88	3.31C±0.10	6.0±0.1	2.14C±0.13	187.67C±19.80	2.61C±0.16	
	Mrigal	1.03±0.02		3.28C±0.13	5.7±0.1	2.25C±0.12	217.20C±8.30	2.88C±0.06	
4	Catla	1.45±0.06		4.78B±0.11	6.7±0.06	3.33B±0.15	234.26B±20.14	2.99A±0.15	1.73
	Rohu	1.17±0.05	90	4.33B±0.12	7.1±0.07	3.16B±0.13	275.00B±1.93	3.28B±0.13	
	Mrigal	1.03±0.02		3.95B±0.13	6.8±0.07	2.90B±0.11	281.97B±7.48	3.35B±0.04	
5	Catla	1.45±0.06		5.58A±0.20	6.8±0.08	4.13A±0.22	290.26A±26.30	3.38A±0.16	1.44
	Rohu	1.17±0.05	93	5.21A±.08	7.5±0.08	4.04A±0.11	352.00A±25.69	3.75A±0.13	
	Mrigal	1.03±0.02		4.38A±0.12	6.9±0.08	3.35A±0.11	324.85A±13.19	3.61A±0.07	

All values are mean±SE of mean. Mean with the same letters in the same column are not significant ($P > 0.05$) different
 SGR (%BW d⁻¹) = Specific growth rate = $[(\ln W_{tf} - \ln W_{ti}) / t] \times 100$.
 Growth per cent gain in body wt. = $[(W_{tf} - W_{ti}) / W_{ti}] \times 100$, where, W_{ti} and W_{tf} denotes initial and final weight of fish respectively, and t represents time (days), duration of experiment (40 days). For details on dietary treatments (1-5) see table 1.
 BW = Body weight, d=day

to 6.0 ± 0.33 . The pH was alkaline (8.08 ± 0.33 to 8.64 ± 0.02). Free carbon dioxide (Co2) was initially absent (20 day sampling) and thereafter only low levels were observed in ponds where the fry were fed on low protein diets. Total alkalinity, carbonates and bicarbonates, hardness, total dissolved solids (TDS) increased with an increase in the protein levels of the diets and also with respect to time. No definite trend in calcium and magnesium levels were observed, though their concentration in the pond waters remained high (Table 4).

Table 4. Effect of supplementary diets (1-5) on physico-chemical and biological characteristics of pond water

Parameters	Treatments (20 days)				
	1	2	3	4	5
Dissolved oxygen mg l ⁻¹	6.0±0.19	6.9±0.11	7.6±0.0	5.7±0.11	6.5±0.11
BOD5 mg l ⁻¹	2.8±0.28	4.2±0.14	4.8±0.28	3.6±0.0	3.6±0.35
pH	8.29±0.01	8.18±0.01	8.64±0.02	8.64±0.01	8.42±0.01
Free CO ₂ mg l ⁻¹	-	-	-	-	-
Carbonates mg l ⁻¹	5.3±1.08	4.0±0.0	10.0±0.94	11.3±1.08	12.0±0.94
Bicarbonates mg l ⁻¹	127.3±1.96	142.6±2.88	133.3±3.31	141.3±6.63	164.6±3.57
Total alkalinity mg l ⁻¹	132.6±3.03	146.6±2.88	143.3±2.37	152.6±6.72	176.6±2.72
Total hardness mg l ⁻¹	466.7±14.4	466.7±27.24	493.3±5.44	496.7±7.20	517.3±3.03
Calcium mg l ⁻¹	52.7±1.50	33.4±0.23	36.4±2.29	37.6±0.22	29.2±1.83
Magnesium mg l ⁻¹	80.8±3.89	93.2±6.69	97.8±2.71	74.4±1.86	100.9±2.43
Chlorides mg l ⁻¹	200.3±4.05	210.0±1.35	216.6±2.34	186.8±7.53	190.1±8.82
Salinity (%)	0.5±0.0	0.6±0.0	0.5±0.0	0.46±0.23	0.5±0.0
Conductivity µScm ⁻¹	1411.3±10.72	1409.3±20.9	1396.7±26.45	1416.2±16.43	1462.7±20.65
Turbidity NTU	72.3±2.19	72.8±0.48	75.7±2.74	75.5±2.60	76.3±6.81
TDS mg l ⁻¹	1200.0±60.0	1333.33±108.99	1400.0±40.0	1483.3±130.62	1733.3±288.37
O-PO ₄ mg l ⁻¹	0.10±0.24	0.09±0.01	0.15±0.0	0.12±0.01	0.21±0.01
Total kjeldahl Nitrogen mg l ⁻¹	6.21±0.39	7.05±0.12	8.83±0.09	14.10±0.12	14.40±0.21
NO ₃ -N mg l ⁻¹	3.70±0.38	5.35±0.10	5.25±0.75	5.60±0.33	11.40±0.50
NO ₂ -N mg l ⁻¹	0.11±0.01	0.05±0.0	0.16±0.01	0.18±0.01	0.1±0.0
NH ₄ -N mg l ⁻¹	0.87±0.01	0.78±0.0	0.68±0.04	0.57±0.02	0.47±0.01
NPP mg C l ⁻¹ d ⁻¹	0.3±0.0	0.3±0.70	0.33±0.01	0.21±0.04	0.51±0.01
GPP mg C l ⁻¹ d ⁻¹	1.40±0.10	1.40±0.0	2.20±0.04	2.02±0.14	2.37±0.01
Chlorophyll 'a' mg l ⁻¹	0.53±0.01	1.19±0.01	1.77±0.11	1.82±0.02	1.91±0.02
Phytoplankton nos l ⁻¹	2336±272.3	2380±226.9	3200±148.9	2750±262.4	2570±156.02
Zooplankton nos l ⁻¹	290±92.19	660±212.80	1220±248.20	270±35.46	710±269.50
Phytoplankton (d)	1.69±0.13	1.56±0.24	1.42±0.08	1.92±0.02	1.95±0.02
Zooplankton (d)	1.18±0.26	1.55±0.01	1.16±0.12	1.17±0.25	1.89±0.01
	Treatments (40 days)				
	1	2	3	4	5
Dissolved oxygen mg l ⁻¹	6.2±0.11	6.73±0.17	6.83±0.18	7.13±0.08	7.56±0.03
BOD5 mg l ⁻¹	6.0±0.19	5.3±0.11	5.0±0.11	5.8±0.67	6.0±0.32
pH	8.18±0.01	8.08±0.33	8.32±0.02	8.21±0.04	8.38±0.01
Free CO ₂ mg l ⁻¹	1.03±0.02	1.03±0.02	0.0	0.0	0.0
Carbonates mg l ⁻¹	-	-	4.66±0.54	13.33±2.72	24.00±1.88
Bicarbonates mg l ⁻¹	133.33±2.72	149.33±1.55	145.33±1.54	140.0±13.63	193.33±14.17
Total alkalinity mg l ⁻¹	133.33±2.72	149.33±1.55	150.7±1.54	155.3±13.44	215.3±15.83
Total hardness mg l ⁻¹	2183.3±36.04	2333.3±19.65	2393.3±14.41	2350.0±47.19	2506.7±30.34
Calcium mg l ⁻¹	59.4±0.45	50.5±3.17	55.5±1.37	53.5±1.27	62.8±1.65
Magnesium mg l ⁻¹	494.5±9.02	536.4±6.30	547.9±4.17	538.6±10.87	511.0±6.60
Chlorides mg l ⁻¹	291.0±25.79	286.0±21.24	332.3±40.54	375.3±11.55	462.9±6.75
Salinity (%)	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0
Conductivity µScm ⁻¹	1474.2±23.33	1553.2±19.06	1484.5±21.35	1482.5±28.21	1500.3±32.71
Turbidity NTU	84.0±3.69	113.3±0.27	145.0±2.63	147.3±2.68	161.0±8.22
TDS mg l ⁻¹	933.3±288.37	1200±188.78	1600±0.00	1733.3±217.99	2400.0±188.78
O-PO ₄ mg l ⁻¹	0.10±0.00	0.08±0.02	0.10±0.01	0.12±0.01	0.12±0.00
Total kjeldahl Nitrogen mg l ⁻¹	4.36±0.03	5.86±0.21	8.7±0.31	11.5±0.13	13.8±0.11
NO ₃ -N mg l ⁻¹	5.83±0.19	5.77±0.26	7.11±0.26	8.75±0.25	9.56±0.46
NO ₂ -N mg l ⁻¹	0.45±0.03	0.25±0.01	0.23±0.01	0.19±0.02	0.16±0.01
NH ₄ -N mg l ⁻¹	0.67±0.02	0.66±0.01	0.43±0.02	0.45±0.02	0.37±0.01
NPP mg C l ⁻¹ d ⁻¹	0.25±0.04	0.45±0.07	0.40±0.04	0.50±0.04	0.90±0.14
GPP mg C l ⁻¹ d ⁻¹	1.10±0.04	1.10±0.04	1.60±0.11	2.15±0.16	3.50±0.08
Chlorophyll 'a' mg l ⁻¹	1.17±0.01	1.07±0.01	0.91±0.02	1.07±0.01	1.28±0.02
Phytoplankton nos l ⁻¹	3810±80.23	2930±25.59	2790±47.19	4720±130.79	4520±118.02
Zooplankton nos l ⁻¹	870±70.79	670±42.48	1300±23.30	1010±28.31	1470±4.71
Phytoplankton (d)	2.26±0.01	2.09±0.01	2.25±0.01	2.19±0.01	2.27±0.01
Zooplankton (d)	1.74±0.08	1.36±0.01	1.56±0.01	1.52±0.01	1.95±0.01

All values are mean ± SE of mean; Temperature during the experimental period ranged between 27.1 to 31.7°C; For details on dietary treatments (1-5) see table 1.

Nutrients and primary productivity

In general o-Po_4 , total kjeldhal nitrogen and $\text{No}_3\text{-N}$ increased, where as $\text{NH}_4\text{-N}$ levels decreased with an increase in the protein contents (Treatments 3 to 5). Significantly ($P < 0.05$) highest $\text{NH}_4\text{-N}$ levels were observed in fish ponds where the fry were fed on diets containing only MOC and rice-bran and also along with fish meal (Treatments 1 and 2). Similarly, indicators of primary productivity viz. chlorophyll 'a', net primary productivity (NPP) and gross primary productivity (GPP) also an increased with increase in the protein contents of the diets, and thus highest values in these parameters were observed in treatment number 5 (Table 4).

Plankton community and species diversity

The abundance of phyto and zooplanktons associated with different dietary treatments are shown in table 4 and figures 2 to 3. Though no definite trend in plankton population was observed, however, their number increased with respect to time. Plankton population was significantly

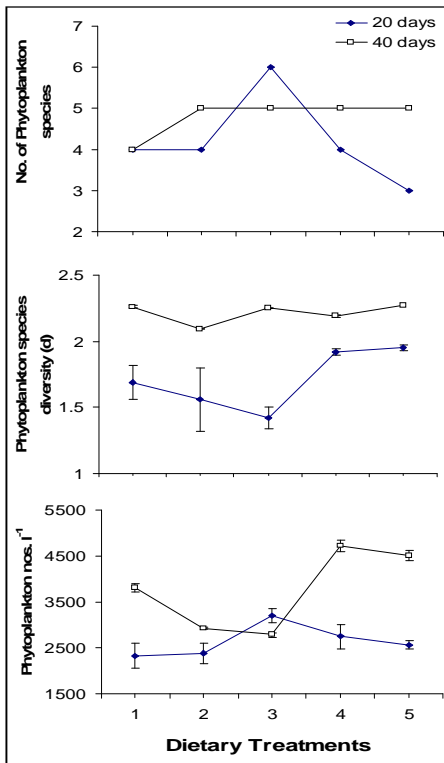


Fig. 2. Total population (Nos. l⁻¹), species diversity (d) and number of species of phytoplankton at five different dietary treatments (1-5): 1 = 28.1% protein; 2 = 32.6% protein; 3 = 32.2% protein; 4 = 33.7% protein; 5 = 35.15% protein

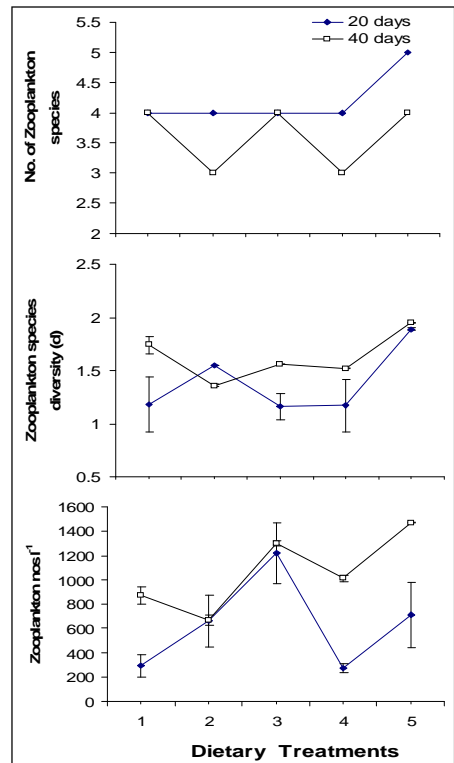


Fig. 3. Total population (Nos. l⁻¹), species diversity (d) and number of species of zooplankton at five different dietary treatments (1-5): 1 = 28.1% protein; 2 = 32.6% protein; 3 = 32.2% protein; 4 = 33.7% protein; 5 = 35.15% protein

high in treatments 4 and 5 where the fry were fed on high protein (34 to 35%) diets. Abundance of different phytoplankton taxa indicated that bacillariophyceae (5 taxa) dominated the community, followed by chlorophyceae (3 taxa). Cyanophyceae was represented by only one taxa *Gomphosphaeria*, which appeared in ponds where the fry were fed on diets containing 34 to 35% protein. As far as zooplankton abundance is concerned, copepoda dominated the community and was represented by cyclops and nauplius. Cyclops were common to all the treatments and their numbers increased with an increase in the protein levels of the diets. Rotifera was represented by three taxa viz. *Brachionus*, *Keratella* and *Kellicotia*, whereas cladocera was represented by *Daphnia* and found only in the 5th treatment.

Highest species diversity of both phyto and zooplankton was also found to be associated with the highest protein levels of the diets (Table 4, Figs. 2 and 3).

Sediment characteristics

The pH of the sediment was more or less similar in all the treatments and fluctuated between 7.09 ± 0.002 to 7.78 ± 0.01 (Table 5). Alkalinity, hardness, o-Po₄, No₃-N increased with an increase in the protein levels of the diets. EC and chlorides did not exhibit any definite trend. Though, no definite trend in organic carbon was observed, however, in general the values decreased with an increase in the protein levels of the diets.

Benthic population also increased significantly ($P < 0.05$) with an increase in the protein levels of the diets and was represented by oligochaeta (2 taxa), mollusca (1) and diptera (1 taxa).

Discussion

A significant ($P < 0.05$) decrease in FCR, increase in survival and growth in terms of live weight gain, SGR and growth per cent gain in body was observed in ponds, where the fry were fed on a diet containing 35% protein from processed full fat soybean. On the other hand, low growth and survival were observed in fish fry fed on traditional diets (Treatments 1 and 2) used by the farmers during various culture operations.

A similar level of dietary protein requirements for maximum growth has also been reported for *Cirrhinus mrigala* (Das and Ray 1991). *Cyprinus carpio* (Ogino and Saito 1970, Lieder et al. 1990) and for the climbing perch, *Anabas testudineus* (Patra and Ray 1988). On the other hand, Singh et al. (1987) had reported that the fry of *C. mrigala* require about 45% protein and any further increase in the protein levels resulted to reduction in growth. Recent studies of this laboratory (Kalla 2002) have also revealed that the optimum protein requirements of *Cirrhinus mrigala* fingerlings is about 40% when processed full fat soybean was used as the protein source in the supplementary diets and any further increase in protein levels

Table 5. Effect of supplementary diets (1-5) on physico-chemical and biological characteristics of pond sediment

Parameters	Treatments (20 days)				
	1	2	3	4	5
Moisture (%)	27.97 ±0.0	25.05 ±0.0	26.84 ±0.0	27.61 ±0.0	24.08 ±0.0
Conductivity m Scm ⁻¹	293.5 ±1.19	459.0 ±3.31	203.0 ±0.471	213.0 ±0.47	205.0 ±2.35
pH	7.29 ±0.002	7.20 ±0.004	7.47 ±0.002	7.67 ±0.007	7.70 ±0.002
Alkalinity mg g ⁻¹	0.25 ±0.01	0.24 ±0.05	0.22 ±0.002	0.21 ±0.005	0.26 ±0.005
Hardness mg g ⁻¹	0.22 ±0.01	0.20 ±0.001	0.23 ±0.01	0.18 ±0.01	0.14 ±0.005
Phosphate mg g ⁻¹	0.03 ±0.002	0.033 ±0.004	0.31 ±0.01	0.025 ±0.0	0.022 ±0.002
Nitrate mg g ⁻¹	0.028 ±0.005	0.027 ±0.005	0.026 ±0.002	0.035 ±0.01	0.032 ±0.01
Chlorides mg g ⁻¹	1.47 ±0.01	1.17 ±0.01	0.86 ±0.02	1.16 ±0.03	0.58 ±0.03
Organic matter (%)	0.50 ±0.76	2.57 ±0.14	1.59 ±0.19	2.27 ±0.11	1.64 ±0.55
Benthos m ⁻²	326.0 ±0.10	489.0 ±8.0	419.0 ±20.0	326.0 ±12.0	559.0 ±26.0
Species diversity (\bar{d})	0.80 ±0.01	0.98 ±0.01	0.99 ±0.01	0.93 ±0.01	0.99 ±0.01
	Treatments (40 days)				
	1	2	3	4	5
Moisture (%)	24.29 ±0.0	29.46 ±0.0	27.66 ±0.0	26.32 ±0.0	31.21 ±0.0
Conductivity m Scm ⁻¹	203.5 ±0.27	539.5 ±0.27	196.5 ±0.72	213.0 ±1.42	203.0 ±0.0
pH	7.10 ±0.03	8.56 ±0.0	7.78 ±0.01	7.59 ±0.01	7.63 ±0.01
Alkalinity mg g ⁻¹	0.30 ±0.03	0.56 ±0.02	0.65 ±0.08	0.78 ±0.03	0.89 ±0.01
Hardness mg g ⁻¹	0.49 ±0.01	0.75 ±0.01	0.76 ±0.016	0.83 ±0.03	1.10 ±0.02
Phosphate mg g ⁻¹	0.023 ±0.001	0.034 ±0.001	0.043 ±0.001	0.052 ±0.0	0.056 ±0.01
Nitrate mg g ⁻¹	0.025 ±0.001	0.033 ±0.01	0.036 ±0.002	0.04 ±0.01	0.044 ±0.01
Chlorides mg g ⁻¹	0.81 ±0.10	0.89 ±0.04	0.75 ±0.0	0.67 ±0.03	0.38 ±0.02
Organic matter (%)	0.91 ±0.03	1.66 ±0.11	1.95 ±0.062	1.21 ±0.16	0.55 ±0.13
Benthos m ⁻²	278.0 ±8.0	350.0 ±10.0	512.0 ±10.0	536.0 ±0.18	629.0 ±24.0
Species diversity (\bar{d})	0.80 ±0.02	0.97 ±0.0	1.0 ±0.01	0.98 ±0.01	0.99 ±0.01

All values are mean± SE of mean. For details of dietary treatments (1-5) see table 1

repressed growth. In spite of the fact that ponds were rich with planktonic flora and fauna, live weight gain, SGR and growth per cent gain in body weight appeared to be a function of supplementary diets only. Since survival

and growth increased with each increase in the dietary protein level, it indicated that the dietary protein requirements of the Indian major carp fry is higher than 35% and the essential substances available in natural food are perhaps not sufficient enough to supply the required nutrients to the growing fish, which perhaps may be attributed to the high stocking density. Further, independent of the presence of natural food, diets of high value bring good conditions which enable further raising at excellent survival rates (Steffens 1990).

Marked variations in the water quality parameters as a result of feeding the fry on supplementary diets indicate a differential response in nutrient release and productivity indicating parameters. Dissolved oxygen (DO) levels were at optimal levels in all the treatments and thus DO was not a limiting factor and it showed a significant and positive correlation with fish weight gain ($r=0.782$), SGR ($r=0.692$) and growth per cent gain in body weight ($r=0.696$). On the other hand DO showed a significant negative correlation with $\text{NH}_4\text{-N}$ ($r=-0.807$), indicating that high DO favors growth, while high $\text{NH}_4\text{-N}$ suppressed growth. A significant negative correlation of $\text{NH}_4\text{-N}$ with SGR ($r=-0.693$) further corroborate the results. Free carbon dioxide was either absent or only low levels were detected indicating its continuous utilization by the phytoplanktons.

A significant ($P<0.05$) and positive correlation of alkalinity, turbidity, TDS, o-Po_4 , total kjeldahl nitrogen and $\text{No}_3\text{-N}$ with NPP ($r=0.543, 0.599, 0.651, 0.529, 0.719$ and 0.623 , respectively), confirms the high trophic status of the ponds, which are in agreement with those of Almazan and Boyd (1978), Zur (1981), Knud-Hansen and Batterson (1994) and Garg and Bhatnagar (1996, 1999 and 2000).

Present studies further revealed that with an increase in the inclusion levels of soybean in the diets, fry survival and growth improved, while ammonia pollution of water is diminished. Reduction in N-NH_4^+ levels with the use of processed soybean in fish diets have also been reported by many workers (Deepak 1999, Priyanka 1999, Kalla et al. 2001) and these observations are very important for developing high density culture system.

The fluctuating pattern of phytoplankton abundance could be attributed to the grazing pressure exerted by the zooplankton (Wade and Stirling 1990), while variations in zooplankton community may be attributed to the changing pattern of food resources availability of phytoplankton (Gulati 1983, Karabin 1985, Little and Muir 1987). A significant and positive correlation of zooplankton with phytoplankton ($r=0.869$), NPP ($r=0.502$), fish weight gain ($r=0.635$), SGR ($r=0.628$) and significant positive correlation of the productivity indicating parameters, viz. alkalinity, NPP, GPP and chlorophyll 'a' with fish weight gain ($r=0.641, 0.624, 0.884$ and 0.388 respectively) may also indicate that increase in fish biomass can also be reasonably attributed to the increase in productivities of nurseries perhaps as a result of supplementary feeding. Liang et al. (1981); Olah et al. (1986), Teichert-coddington (1986), Garg and Bhatnagar (1996, 1999 and 2000) have also advocated the positive correlation of primary productivity with fish growth/yield.

Sediment chemistry has also revealed that the ponds where the fish were fed on high protein diets became successively richer in o-Po4 and nitrogen. The number of macrozoobenthos observed in the present studies are related to the degree of nutrient enrichment revealing a significant and positive correlation with sediment alkalinity ($r=0.936$), o-Po4 ($r=0.877$), No3-N ($r=0.941$) and also with the dietary protein ($r=0.857$) levels. These observations are similar to those of Stirling and Wahab (1990). Decrease in organic carbon contents with increase in growth and survival may be attributed to the continuous consumption of feed by the growing fish.

High benthic population in the nurseries where the fish were fed on high protein diets may be attributed to their non-consumption by the young fish (Steffens 1990). Increase in macrozoobenthos especially *Tubifex* (oligochaetae) was also found to be concomitant with the nutrient status of the ponds. Lennan et al. (1985) and Bardach (1986) were of the view that oligochaets mix soils and pump proportionally large quantities of water into it, which increases pore water exchange, resulting in high nutrient release, advocating a significant and positive correlation of benthic population with nutrients (o-Po4, $r=0.971$; No3-N, $r=0.94$).

These results indicate that in such a high density (32 m²) culture system the importance of natural food decreases, and the use of compounded diets with high protein (>35%) and energy levels are required to raise survival and growth rates of fry. Present studies therefore indicate that the traditional diets are nutritionally inadequate, hence for high survival and growth, fry may have to be fed on supplementary diets containing high protein levels (<35%) even under field conditions. To arrive at a definite conclusion regarding the optimum protein requirements of fry under field conditions, further studies are needed to be conducted.

Acknowledgments

The research work was supported by the state project on fisheries (C(a)Zoo-1 NP Agri.). Authors are also grateful to M/s Royal Fish Farm, Hisar for providing fish farm facilities. We are also grateful to the anonymous reviewer for critically reading the manuscript and making many insightful suggestions for improvement.

References

- Almazan, G. and C.E. Boyd. 1978. Plankton production and tilapia yields in ponds. *Aquaculture* 15: 75-77.
- Alikunhi, K.H. 1952. On the food of young carp fry. *Journal of Zoological Society of India* 4: 77-84.
- Alikunhi, K.H. and V. Ramchandran. 1955. On the mortality of carp fry in nursery ponds and role of plankton in their survival and growth. *Indian Journal of Fisheries* 2 (2): 257-313.
- AOAC (Association of official Analytical Chemists), 1995. Official methods of analysis. Assoc. off. Anal. Chem. Inc., Arlington, USA, 684pp.

- APHA (American Public Health Association), 1998. Standard methods for the examination of water and waste water. APHA, AWWA, WPCF, 16Ed, New York.
- Bardach, J.E. 1986. Constraints of Aquaculture. *Aquaculture Engineering* 5: 287-300.
- Boyd, C.E. 1992. Water quality management for pond fish culture. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands. 316pp.
- Das, I. and A.K. Ray. 1991. Growth response and feed conversion efficiency in *Cirrhinus mrigala* (Ham.) fingerlings at varying dietary protein levels. *Journal of Aquaculture in the Tropics* 6: 179-185.
- Deepak, 1999. Effect of processed soybean and mineral mixture on digestibility and growth parameter in catfish, *Heteropneustes fossilis* (Bloch) for sustainable culture. M.Sc. Thesis submitted to Chowdhary Charan Singh Haryana Agricultural University, Hisar, pp. 1-96.
- Duncan, D.B. 1955. Multiple range and multiple F-tests. *Biometrics* 11: 1-42.
- Garg, S.K. and A. Bhatnagar. 1996. Effect of varying doses of organic and inorganic fertilizers on plankton production and fish biomass in brackishwater fish ponds. *Aquaculture Research* 27: 157-166.
- Garg, S.K. and A. Bhatnagar. 1999. Effect of different doses of organic fertilizer (cow-dung) on pond productivity and fish biomass in still water ponds. *Journal of Applied Ichthyology* 15: 10-18.
- Garg, S.K. and A. Bhatnagar. 2000. Effect of fertilization frequency on pond productivity and fish biomass in still water ponds stocked with *Cirrhinus mrigala* (Ham.). *Aquaculture Research* 31: 409-414.
- Gulati, R.D. 1983. Zooplankton and its grazing as indicators of trophic status in Dutch lakes. *Environmental Monitoring and Assessment* 3: 34-354.
- Hovgaard, P. 1973. A new system of sieving for benthic samples. *Sarsia* 53: 13-15.
- Jhingran, V.G. and R.S.V. Pullin. 1988. A hatchery manual for the common, chinese and Indian major carps. Asian development bank, International Centre for living aquatic resources management (ICLARM), Philippines, pp. 163.
- Kalla, A. 2002. Effects of supplementary feeding in some teleosts on growth, digestibility and water quality parameters in intensive fish culture system. Ph.D. Thesis Guru Jambheshwar University, Hisar, India, pp. 1-230.
- Kalla, Alok, C.P. Kaushik, S.K. Garg and Anita Bhatnagar. 2001. Role of plant protein in Sustainable Aquaculture. In: NBFGR publication No. 3. Captive breeding for aquaculture and fish germplasm conservation (Ponniah, A.G., Lal, K.K. and Basheer, V.S. eds.) pp.1-2.
- Karabin, A. 1985. Pelagic zooplankton (Rotatoria+Crustacea) variation in the process of lake eutrophication. 1. Structural and quantitative features. *Ekologia Polska* 33(4): 567-616.
- Kund-Hansen, C.F. and T.R. Batterson. 1994. Effect of fertilization frequency on the production of Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 123: 271-280.
- Lennan, J.E., R. o'Neal Smitherman and G. Tchobonoglous (eds.) 1985. Principles and practices of pond aquaculture, oregon state University, U.S.A: 252 pp.
- Liang, Y., J.M. Maleck and J. Wang. 1981. Primary production and fish yields in Chinese ponds and lakes. *Transaction of the American Fisheries Society* 110: 346-350.
- Lieder, U., W. Steffens and B. Rennert. 1990. Zur Charakteristik der Mischfuttermittel für die Karpfenproduktion und über perspektiven ihrer weiteren Entwicklung. *Fortschr Fischereiwiss* 9.
- Little, D. and J. Muir. 1987. A guide to integrated warm water aquaculture institute of Aquaculture, University of Stirling, 238 pp.
- Mitra, G.N. and P. Mohapatra. 1956. on the role of zooplankton in the nutrition of carp fry. *Indian Journal of Fisheries* 3(2): 299-310.
- Needham, J.G. and P.R. Needham. 1963. A guide to the study of fresh water Biology. Holden-Day, Inc. San Francisco, U.S.A: 102 pp.
- Ogino, C. and K. Saito. 1970. Protein nutrition in fish.I. The utilization of dietary protein by young carp. *Bulletin of Japanese Society of Science and Fisheries* 36: 250-254.
- Olah, J., V.R.P. Sinha, S. Ayyappan, C.S. Purushothaman and S. Radheysyam. 1986. Primary production and fish yields in fish ponds under different management practices. *Aquaculture* 58: 111-122.
- Patra, B.C. and A.K. Ray. 1988. Influence of dietary protein source on the protease activity, protein synthesis and certain biochemical composition of the flesh in *Anabas testudineus* (Bloch). In: M.Mohan Joseph (Ed.). The First Indian Fisheries Forum, Proceedings. Asian Fisheries Society, Indian Branch, Mangalore, pp. 55-59.
- Piper, C.S. 1950. Soil and plant analysis, Inter Science Publ. New York: 368pp.

- Prien, M., G. Hulata and D. Pauly. 1993. on the use of multivariate statistical methods in aquaculture research. In: M. Prien, G. Hulata and D. Pauly (eds.) *Multivariate methods in Aquaculture Research: case studies of tilapias in experimental and commercial systems*. ICLARM Stud. Rev. 20: 1-2.
- Priyanka, 1999. Evaluation of some plant origin proteins for growth and digestibility in catfish *Heteropneustes fossilis* (Bloch). M.Sc. Thesis submitted to the CCS HAU, Hisar: pp. 1-96.
- Singh, B.N., V.R.P. Sinha and K. Kumar. 1987. Protein requirements of an Indian major carp, *Cirrhinus mrigala* (Ham.). *International Journal of Academy of Ichthyology* 8(1): 71-75.
- Steffens, W. 1990. Interrelationships between natural food and supplementary feeds in pond culture. Proc. of FAo-EIFA Symposium on production enhancement in still-water pond culture, Prague: 218-229.
- Stirling, H.P. and M.A. Wahab. 1990. Benthic ecology and dietary importance of benthos to trout in earthen ponds. Proc. of FAo-EIFAC Symposium on production enhancement in Still-water pond culture, Prague: pp. 48-60.
- Teichert-Coddington, D.R. 1986. Effect of manuring regime and alkalinity on primary production and yield of tilapia using liquid swine manure. Doctoral Dissertation, Auburn University, Dept. of Fisheries and Allied Aquaculture: 73pp.
- Wade, J.W. and H.P. Stirling. 1990. The effects of fertilization on the production of plankton and benthic fauna in relation to Trout culture in earthen ponds. Proc. of FAo-EIFAC symposium on production enhancement in still-water pond culture, Prague: 146-164.
- Ward, H.B. and G.C. Whipple. 1959. *Freshwater Biology*. John Wiley and Sons, 1248 pp.
- Washington, H.G. 1984. Diversity, biotic and similarity indices. *Water Research* 18(6): 653-694.
- Wetzel, R.C. and G.E. Likens. 1979. *Limnological analysis*. Philadelphia, PA, USA, Saunders: 357pp.
- Zur, O. 1981. Primary production in intensive fish ponds and a complete organic carbon balance in the ponds. *Aquaculture* 23: 197-210.