reading between 22.0 and 25.8 and chlorophyll content below 6 mg/g. The lowest TC content of 5.15 mg/g was recorded in 'PBS 14016' with a SPAD reading 22.01.

Correlation coefficients (r) and regression equations between SPAD reading and chlorophyll contents and VCR are presented in Table 2. Correlation coefficients between the SPAD reading and chlorophyll contents were highly significant at all the stages of sampling. Over the mean of four samples recorded at 30, 45, 60 and 75 DAE, the r values between the SPAD reading and chlorophyll *a* (0.94**), chlorophyll *b* (0.90**), and TC content (0.93**) were very high, positive and significant, indicating closer relationship of these traits with the SPAD reading, i.e. higher the SPAD reading higher will be the chlorophyll pigments and vice versa. The regression lines (Figure 2 *a–d*) showed that these variables are linearly related with each other. On the basis of the linear relationship, regression equations were developed (Table 2). Further, to test the consistency in ranking of genotypes with respect to the SPAD reading and estimated chlorophyll contents, the rank correlation coefficients (r_s) were calculated. It was found that the value of r_s between SPAD and chlorophylls *a*, *b* and TC content was very high and positive (0.94, 0.91 and 0.94, respectively), showing similar rankings of different genotypes. In the present investigation, the tolerant genotypes showed SPAD reading more than 30 and total chlorophyll content more than 8.0 mg/g dry weight of leaves and VCR below 2.0. On the other hand, genotypes with SPAD reading below 25, TC content less than 6.0 mg/g, and VCR more than 2.75 were found to be sensitive to iron-chlorosis. On the basis of the regression equations given here, it is convenient for researchers to predict chlorophyll content and VCR in the plants for categorization of genotypes.

The use of the SPAD meter in nitrogen management of tall fescue[8] (*Festuca arundinaceae*) and switch grass[9] (*Panicum virgatum*) has been demonstrated. The present study clearly shows that the chlorophyll meter (SPAD) is an efficient and speedy equipment for chlorophyll estimation in groundnut and can be used for screening genotypes having higher efficiency of iron utilization and thus selecting iron-chlorosis tolerant genotypes.

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Replacement of live-food with refrigerated-plankton food for *Cyprinus carpio* (L.) larvae cultured with three different types of biological filters

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Common carp, *Cyprinus carpio* (L.) larvae were cultured with either live-food or refrigerated-plankton food in the recirculating system. Three types of biological filters: (i) pebbles, foam and weed (*Lemna major*) filter system (PFWS), (ii) pebbles and foam filter system (PFS), and (iii) only weed (*Lemna major*) filter system (WS) were used for each feeding scheme. After 40 days of culture, a significantly higher ($P < 0.01$) rate of survival was obtained with the live-food system (LFS) than the refrigerated-plankton food system (RPFS) regardless of filtration type. The final average weight was influenced by both filtration unit and food. Significantly higher ($P < 0.05$) values for average weight (130 ± 1.5 mg), specific growth rate (4.69 ± 0.01), and RNA/DNA ratio (5.60 ± 0.13) were obtained in the PFWS of live-food treatment. Food was more efficiently utilized in the LFS as indicated by the significantly lower ($P < 0.05$) values of food conversion ratio (0.93 to 1.60) compared to the RPFS (3.87 to 4.91). Amylase (0.09–0.162 mg maltose/mg protein/h) and proteolytic enzyme (2.52–4.70 mg tyrosine/mg protein/h) activities were significantly higher in the LFS than the RPFS. Significantly higher values for ammonia (0.014 ± 0.031 mg/l), phosphate (0.150–0.157 mg/l) and COD (197–207 mg/l) were observed in the PFS than for the other two filter systems. Results indicate that use of *Lemna major* alone or in conjugation with pebbles and foam helped in the maintenance of improved water quality in the culture system which resulted in the better performance of carp larvae.

COMMON carp, *Cyprinus carpio* (L.) is an important commercial fish in India and is widely used for compos-

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ite culture. A multidisciplinary approach, such as a study of digestive physiology and zootechnical performance, is essential to gain complete control over larviculture as well as for sustainable aquaculture development. The present investigation aims to study the effect of live-food and refrigerated-plankton food on the performance of common carp larvae and on the maintenance of water quality with three different biological filtration units over a 40-day culture period.

Carp larvae (6-day-old, 1.2 ± 0.07 mg) were cultured with two feeding regimes, the live-food system (LFS) and the refrigerated-plankton food system (RPFS) and with three types of biological filters – (i) pebbles, foam and weeds system (*Lemna major*) (PFWS), (ii) pebbles and foam system (PFS) and (iii) weeds system (*Lemna major*) (WS) in recirculating system. The recirculating system consisted of two parts: the upper part had squarish glass aquaria (15 l each) and the lower part had a bigger (55 l) rectangular glass aquarium. The lower part was divided into two compartments of 45 l and 10 l by a glass partition. A biological filter was used in the first, bigger chamber and through a channel the filtered water was brought into the smaller chamber from where it was pumped into the fish culture units with the help of a water pump.

In the PFS, a 20 cm layer of pebbles was overlaid by a 20 cm layer of foam (400 mm). The two layers were separated by a nylon net. In the PFW system, an additional bed of *Lemna major* (30 cm × 45 cm, 100 *g* wet weight) was kept under the pebbles and foam layers. In the third one, only a layer of *Lemna major* (30 cm × 45 cm, 100 *g* wet weight) was maintained. Water flow rate used in the fish aquarium was 0.188 l/min. The duration of water circulation was 6 h per day.

Larvae were stocked at the rate of 125/15 l aquarium. Three replicates were maintained for each group (3 replicates × 3 types of filters × 2 types of food). In the LFS, plankton was supplied at the rate of 120 mg/aquarium (dry weight). In the RPFS, the same amount of plankton was kept in the refrigerator at 4°C for 12 h and then directly supplied to the larvae. Qualitative analysis of plankton samples showed that *Ceriodaphnia* spp. was the dominant species contributing about 70% of the total plankton population. Other species were *Mesocyclops* spp. (10%), *Brachionus* spp. (18%) and the rest were phytoplankton. Water quality parameters were monitored at 10-day intervals throughout the experimental period. Water temperature ranged from 23 to 25.5°C throughout the culture period. The pH of water was measured with a pH meter (HANNA model Hi 8424). Dissolved oxygen was determined with a dissolved oxygen meter (Orion model 810). Ammonia, nitrite, phosphate and COD were measured according to standard methods[1]. After 40 days of growing, larvae were harvested, counted and weighed. In order to study the amylase and proteolytic activity, larvae were sampled at 8 am before morning feeding at the end of the experiment (after 40 days). One larva was sacrificed for each sample. Three replicates were used for each treatment. Dissections under microscope were conducted on a glass plate maintained at 0°C. The digestive tract was homogenized in 10 volumes (v/w) of ice-cold distilled water and centrifuged at 4°C at 10,000 *g*. The supernatants were taken for analysis.

Amylase activity was assayed according to the method in which the increase in reducing power of buffered starch solution is measured[2]. The incubation mixture consisted of 1 ml of 10% starch solution, 1 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml of 10% NaCl and 1 ml of enzyme solution. After 1 h of incubation, reaction was stopped by the addition of 0.5 ml 3,5-dinitro-salicyclic acid and absorbance was measured at 540 nm. Amylase activity was expressed in terms of mg of maltose librated per mg of tissue protein per h at 37°C.

Proteolytic enzyme activity was measured by using a casein substrate[3,4]. The reaction mixture consisted of 1 ml of substrate solution, 1 ml of 0.1 M phosphate buffer (pH 7.6), 1 ml of calcium chloride and 1 ml of crude enzyme extract. Reaction was stopped after 1 h of incubation with 3 ml of 5% TCA solution. After standing for 10 min, the precipitate was removed by centrifugation and the supernatant was stained with diluted Folin–ciocalteu reagent and absorbance was measured at 650 nm. The results were calculated from a standard curve of tyrosine. Protein was estimated by the standard method[5]. The results are given in mg of tyrosine per mg of protein per h at 37°C as the specific activity. The nucleic acids were determined by pentose analysis[6]. RNA and DNA were determined by orcinol reaction and diphenylamine reaction, respectively.

Food conversion ratio (FCR) and the specific growth rate (SGR) were calculated according to the following equations: $\mathrm{FCR} = W_{dt}/(W_2 - W_t)$; $\mathrm{SGR} = 100 \times (I_n W_2 - I_n W_1)/t$, where W_1 and W_2 are the initial and final weights of larvae, W_{dt} is the dry weight of food and t is the time in days. Differences in fish growth, survival, enzyme activity and water quality parameters were evaluated by analysis of variance, least square difference test and regression analysis (with a computer using the SPSS programme). The level of significance was accepted at $P < 0.05$.

Water temperature ranged from 23 to 25.5°C throughout the culture period. The pH gradually increased over time in all culture systems except the PFWS of the LFS (Figure 1 *a*). Significantly higher ($P < 0.05$) level of dissolved oxygen was observed in the LFS than the RPFS regardless of filtration units. Highest mean value for ammonia (0.031 ± 0.012 mg/l) was obtained in the PFS of the RPFS. Nitrite levels were significantly higher ($P < 0.05$) in the beginning of the study than dur-

ing the remaining culture period (Figure 1 *b*) regardless of culture systems and food. Unlike nitrites, values for phosphate and COD were minimum in the beginning of the study and increased over time (Figure 1 *b*). Mean values of phosphate were significantly higher ($P < 0.05$) in the PFS of both feeding schemes (LFS: 0.150 ± 0.024 mg/l, RPFS: 0.157 ± 0.024 mg/l). Similarly, mean COD level was significantly higher ($P < 0.05$) in the PFS (197 ± 35 to 207 ± 41 mg/l) of both feeding regimes than the remaining systems.

There was no significant difference ($P > 0.05$) in the survival of *Cyprinus carpio* larvae among the PFWS and WS of the LFS as well as PFWS and WS of the RPFS. Highest rate of survival (85%) was obtained in PFWS and WS of the live-food fed group (Table 1). Significantly higher ($P < 0.01$) average weight (130 ± 1.5 mg) was obtained in the PFWS of LFS. Food conversion ratio showed the minimum and maximum values in the PFWS (0.93 ± 0.01) of LFS and PFS (4.91 ± 0.5) of RPFS, respectively. Highest SGR value was obtained in the PFWS of the LFS (Table 1). RNA/DNA ratio was maximum and minimum in PFWS of the LFS (5.60 ± 0.13) and PFS of the RPFS (3.48 ± 0.05), respectively. RNA/DNA ratio in PFWS of the LFS was 5 to 38% higher than the remaining culture systems.

Amylase activity in the PFS of LFS was significantly ($P < 0.01$) lower than the other two filtration systems of the same feeding scheme. The enzyme activity in the PFWS of the the LFS was 3.5 to 11-fold higher than the RPFS. Similarly, the specific proteolytic activity (4.74 ± 0.24 mg tyrosine/mg protein/h) was significantly higher ($P < 0.05$) in the PFWS of the LFS. Specific proteolytic activity in this treatment was 3.4 to 7-fold higher than the RPFS (Table 1). Both the amylase and proteolytic activities showed direct relationship (Figure 2 *a* and *b*) with the average weight of fish regardless of culture systems and feeding regimes.

Feeding of *C. carpio* larvae with live-food led to a better survival rate than the larvae fed with refrigerated-plankton, cultured with similar filtration units in the recirculating systems. The final average weight and the specific growth rate were also significantly ($P < 0.05$) higher in the live-food treatment than the refrigerated one. In African catfish, significantly higher ($P < 0.05$) final mean weight and specific growth rate were obtained in the group fed with live *Artemia* than the group fed with frozen ones, but there was no significant difference ($P > 0.05$) in the survival rate[7]. Asian seabass, *Lates calcarifer* (Bloch) showed significantly higher SGR and survival when fed with live *Moina microcopa* than frozen ones[8]. The denaturation of vitamins and proteins, or lipid oxidation eventually aggravated by the thawing procedure and the thawing duration may explain inferior results obtained with a diet of frozen food organisms. The loss of essential nutrients during thaw-

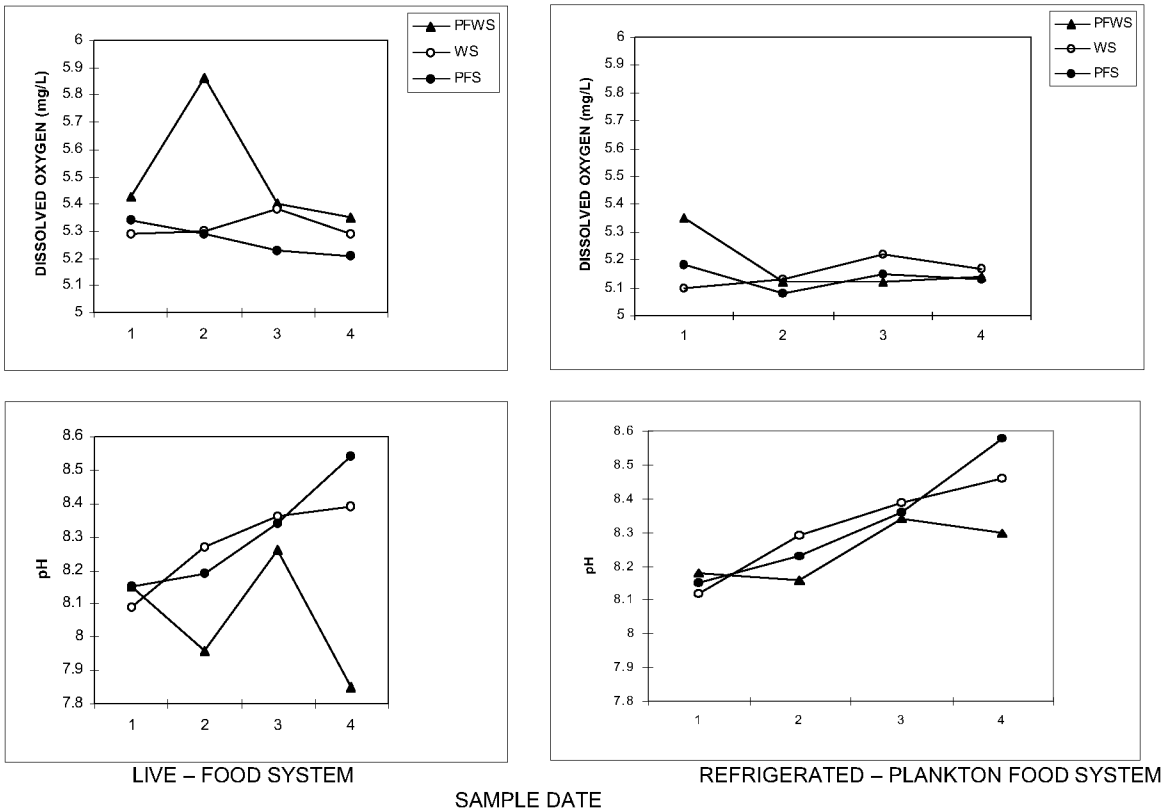

Figure 1 *a*. pH, dissolved oxygen in different culture systems.

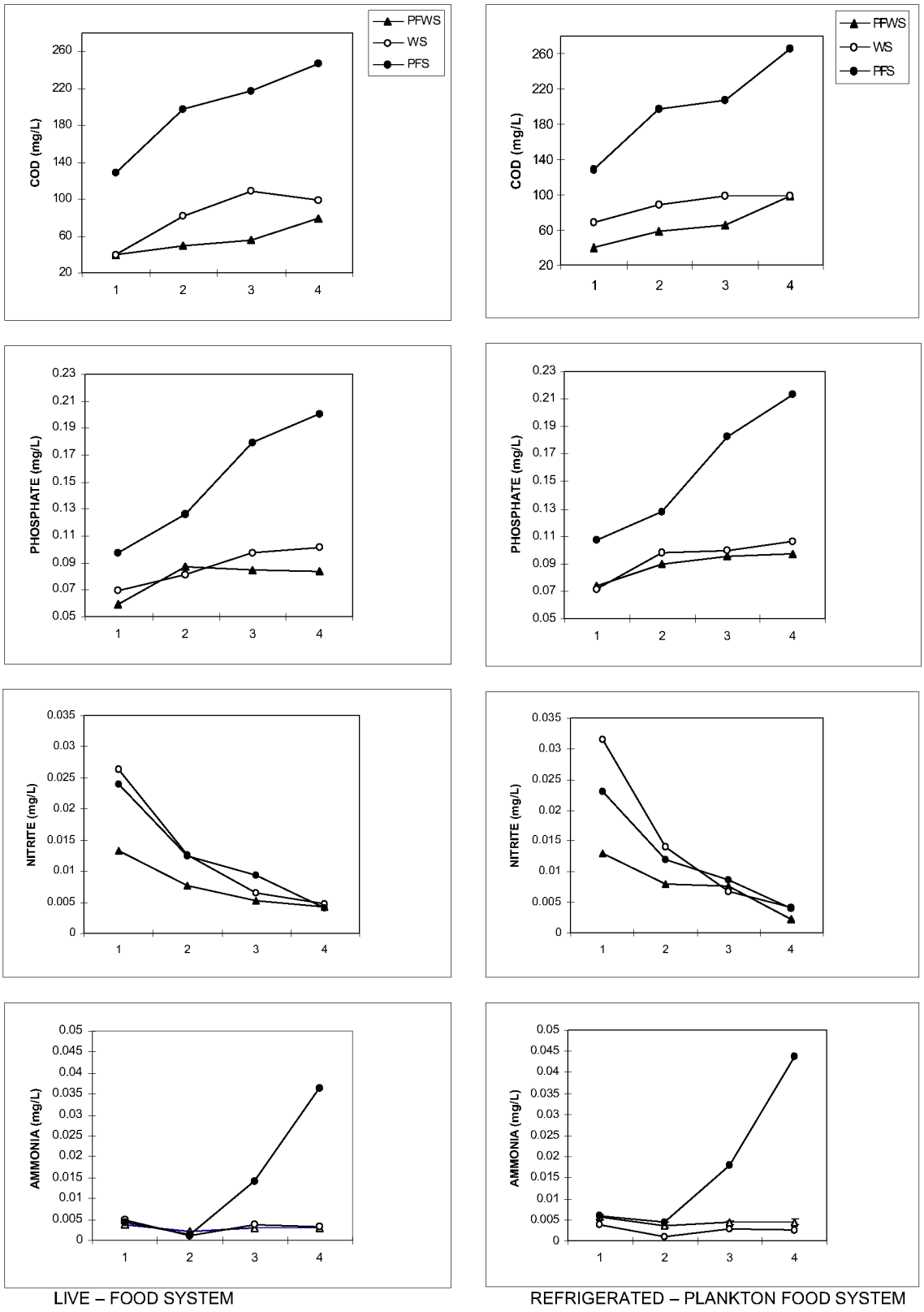

Figure 1 *b*. Mean values for ammonia, nitrite, phosphate and COD in recirculating systems during various days of culture of common carp with three biological systems of two feeding regimes. Each point represents three replicates.

Table 1. Mean and SE of final average weight, percentage survival, feed coversion ratio (FCR), specific growth rate (SGR), RNA/DNA ratio, amylase and proteolytic enzyme activities for common carp fed live-food and refrigerated-plankton food with three types of biological filters in recirculating systems

		PFWS	WS	PFS
Average weight (mg)	LFS	1.130 ± 1.5^{a}	86.33 ± 0.57^{b}	76.33 ± 0.33^{c}
	RPFS	32.33 ± 1.5^{d}	11.29 ± 0.57^{e}	25.67 ± 0.7^{f}
Survival (%)	LFS	11.85 ± 1.45^{a}	11.85 ± 0.33^{a}	11.83 ± 0.7^{b}
	RPFS	11.61 ± 2.33^{c}	11.59 ± 1.20^{c}	11.53 ± 1.53^{d}
FCR	LFS	10.93 ± 0.01^{e}	11.41 ± 0.01^{d}	11.60 ± 0.07^{d}
	RPFS	13.87 ± 0.2^{c}	14.32 ± 0.8^{b}	14.91 ± 0.5^{a}
SGR	LFS	14.69 ± 0.011^{a}	14.29 ± 0.006^{b}	14.18 ± 0.004^{c}
	RPFS	13.35 ± 0.04^{d}	13.25 ± 0.02^{e}	13.13 ± 0.02^{f}
RNA/DNA ratio	LFS	15.60 ± 0.13^{a}	15.33 ± 0.03^{b}	14.90 ± 0.05^{c}
	RPFS	14.27 ± 0.06^{d}	13.90 ± 0.07^{e}	13.48 ± 0.05^{f}
Amylase (mg mal-	LFS	0.162 ± 0.01^{a}	0.150 ± 0.005^{b}	10.09 ± 0.002^{c}
tose/mg protein)	RPFS	0.047 ± 0.008^{d}	10.03 ± 0.007^{e}	0.015 ± 0.003^{f}
Proteolytic enzyme	LFS	14.74 ± 0.24^{a}	13.30 ± 0.08^{b}	12.52 ± 0.21^{c}
(mg tyrosine/mg protein/h)	RPFS	11.41 ± 0.08^{d}	11.08 ± 0.05^{e}	10.69 ± 0.04^{f}

Means not sharing the same letter for each parameter are significantly different ($P < 0.05$).

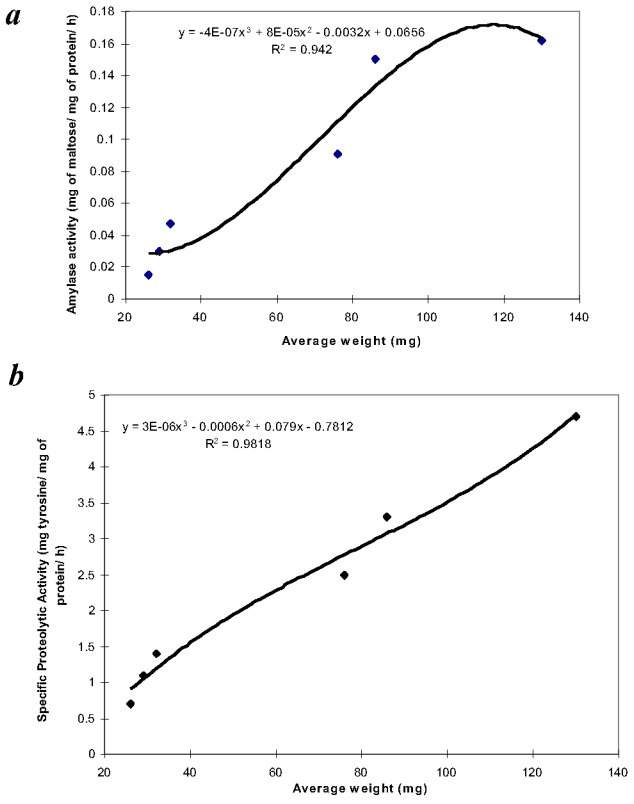

Figure 2. Relationship between ***a***, final average weight and amylase activity; and ***b***, final average weight and specific proteolytic enzyme activity in common carp.

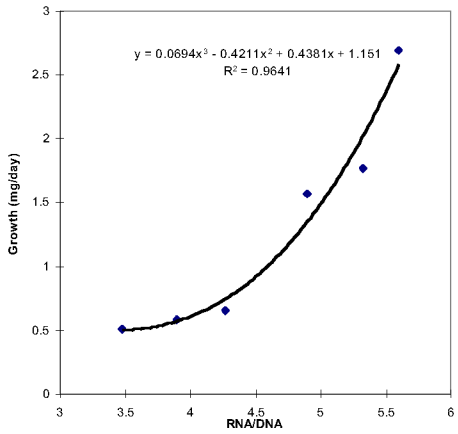

Figure 3. Relationship between RNA/DNA ratio and growth rate (mg/day) of common carp.

ing is probably the most important reason why frozen food organisms have proved to be unsuitable for rearing the larvae of several fish species[9]. Food was more efficiently utilized in the LFS as indicated by a significantly lower ($P < 0.05$) feed conversion ratio compared to the RPFS. This can be explained by significantly higher ($P < 0.05$) amylase and proteolytic activity in the LFS.

Average weight and RNA/DNA ratio of larvae were significantly different ($P < 0.05$) among three filtration systems of both the LFS and RPFS. It seems that survival of larvae was mainly influenced by the quality of food but the weight gain and RNA/DNA ratio were influenced by both the food and the quality of water. RNA/DNA ratio showed a positive relation with growth (Figure 3). Similar results were also obtained in salmonids[10]. Significantly lower ($P < 0.05$) values for ammonia, nitrite, phosphate and COD prevailed in the PFWS which resulted in better performance of larvae in this system. In fish subjected to the stress of high environmental temperatures[11] or heavy metal salts[12], the ratios between these two nucleic acids reduced.

The results of this study indicate that the use of duck weed *Lemna major* as a biological filter in conjugation with the simple pebbles and foam filter helped in the maintenance of water quality in the intensive culture of *C. carpio* larvae which resulted in better performance of

larvae. The results also showed the poor growth of larvae with refrigerated-plankton food. Further study is needed in this regard.

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NMR studies of a neurotoxin (candoxin) from *Bungarus candidus* – Presence of a predominantly b-sheet structure

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Complete sequence-specific ^{1}H resonance assignments of a neurotoxin, candoxin, from *Bungarus candidus* has been reported. Qualitative interpretation of the nOes, chemical shift indices and deuterium exchange rates indicate a highly ordered and rigid conformation for this protein with an extended conformation for more than 50% of the residues and the complete absence of a-helical segments.

TOXICITY of snake venom arises from a complex mixture of ingredients. The toxins target ion channels, receptors and enzymes. One class of these molecules is neurotoxins which bind to cholinergic receptors leading to the closure of the ion channel, thus blocking neurotransmission[1–3]. Neurotoxins can be broadly classified into three groups: short, long and cytotoxins. Short neurotoxins are 58–62 residues long and have four disulfide bridges, while long neurotoxins have 65–74 residues and five disulfide bridges. In both cases the structure consists of three loops protruding from a central core. Most of the sequence heterogeneity among the long neurotoxins is in the first loop and in the C-terminal tail. Structures of several neurotoxins have been solved by crystallographic and NMR methods[4–8]. Though the overall topologies are similar, the function and interaction of each neurotoxin is unique[9]. Recently we have isolated and purified a weak neurotoxin, candoxin, from *Bungarus candidus* (Malayan Krait) venom. Candoxin differs from other known long neurotoxins in that it acts on both pre- and post-synaptic sites.

Here, we describe the complete sequence specific ^{1}H NMR assignments of this toxin and its secondary structure. Protein concentration of 4.5 mM at pH 3.0 (99.9% ^2H_2O and 90% H_2O/10% ^2H_2O) in Shigemi tubes were used wherein smaller volumes are required. NMR experiments carried out on a Varian Unity + 600 MHz NMR spectrometer are: two quantum filtered correlated spectroscopy[10] (2QF-COSY), three quantum filtered correlated spectroscopy[11] (3QF-COSY), clean total correlation spectroscopy[12] (clean TOCSY) ($\blacktriangle_m$ = 80 ms), nuclear Overhauser enhancement spectroscopy[13] (NOESY) ($\blacktriangle_m$ = 50, 75, 150 and 200 ms) and watergate NOESY[14] ($\blacktriangle_m$ = 100, 150 and 200 ms). Relayed COSY[15] was done on a Bruker AMX 500 MHz NMR spectrometer. Deuterium exchange studies were carried out by recording a series of 1D and 2D TOCSY spectra, 12 m after the lyophilized and fully protonated toxin was dissolved in ^2H_2O. Sections of TOCSY and NOESY spectra in the H^α–H^N region are shown in Figures 1 and 2, respectively.

Candoxin has a single polypeptide chain containing 66 amino acid residues with the sequence: MK$\underline{C}^3$KI-$\underline{C}^6$NFDT$\underline{C}^{11}$RAGELKV$\underline{C}^{19}$ ASGEKY$\underline{C}^{26}$FKESWREA-RGTRIERG$\underline{C}^{43}$AAT$\underline{C}^{47}$PKGSVYGLYVL$\underline{C}^{59}\underline{C}^{60}$TTDD $\underline{C}^{65}$N.

In the 1D ^{1}H NMR spectrum, we observe several upfield shifted methyl resonances, downfield shifted H^δ resonances and a highly dispersed H^N region, which indicates a highly ordered and rigid conformation.

Amino acids have been classified on the basis of their side chain spin systems and identified using established procedures[16]. All the five Gly residues could be identified on the basis of their expected H^δ–H^δ connectivity

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